

Program Based Research Grant (PBRG)

Sub-project Completion Report on

**Fortification and Standardization of Nutritional
Level in Selected Human Foods and Efficacy Test
of Polyphenolic Compounds in Livestock
Products**

**Sub-project Duration
14 August 2018 to 31 December 2021**

**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**

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Implementing Organization



Grain Quality & Nutrition Division
Bangladesh Rice Research Institute, Gazipur 1701



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National Agricultural Technology Program-Phase II Project
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Abbreviation and Acronyms

%	Percentage	IBD	Infectious Bursal Disease
μL	Microliter	ICDDR	International Center for Diarrhea Disease Rehabilitation, Bangladesh
°C	Degree Celsius	IDB	International Development Bank
a	Redness	IFAD	International Fund for Agricultural Development
AAC	Apparent Amylose Content	Ig	Immunoglobulin
AAS	Atomic Absorption Spectrophotometer	IG	Ivy Guard
AC	Alternate Current	IG-D	IvyGourd Diet
ADF	Acid Detergent Fibre	Kcal	Kilocalorie
ADG	Average Daily Gain	Kg	Kilogram
AGPs	Antibiotic Growth Promoters	KMS	Potassium Mutebi-Sulphate
AI	Albumin Index	L	Lightness
ALP	Alkaline Phosphatase	LC-MS	Liquid Chromatography Mass Spectrometry
ALT	Alanine Aminotransferase	LG	Lemon Grass
ALT	Alanine Transaminase	LG-D	Lemongrass Diet
AMR	Anti-Microbial Resistance	LWG	Live Weight Gain
ANOVA	Analysis of Variance	MC	Moisture Content
AOAC	Association of Official Agricultural Chemists	mg	Milligram
AST	Aspartate Amino Transaminase	MI	Milk Yield
AWEEC	Animal Welfare and Experimental ethics Committee	mm	Millimeter
b	Yellowness	mmHg	Millimeter of Mercury
β	Beta	MS	Microsoft Excel
		Excel	
BARC	Bangladesh Agricultural Research Council	NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
BARI	Bangladesh Agricultural Research Institute	NATP	National Agriculture Technology Project
BAU	Bangladesh Agricultural University	ND	Newcastle Disease
BAURES	Bangladesh Agricultural University Research System	NDF	Neutral Detergent Fabre
BDT	Bangladeshi Taka	NFE	Nitrogen Free extract
BIDS	Bangladesh Institute of Development Studies	Ni	Nickel
BMI	Body Mass Index	nm	Nanometer
BRR	Bangladesh Rice Research Institute	NMR	Nuclear Magnetic Resonance Spectroscopy
BSTI	Bangladesh Standards and Testing Institute	ω	Omega
BW	Body Weight	OAC	Oil Absorption Capacity
Ca	Calcium	Pb	Lead
CBC	Complete Blood Count	PC	Polyphenolic Compound
Cd	Cadmium	PCR	Project Completion Report
CDC		PHP	Plantain Herb Powder
CF	Crude Fibre	PHTD	Post Harvest Technology Division
CIE	Commission International de l'Eclairage	PI-D	Plantain-Ivy Gourd Diet
CON	Control	PIU	Project Implementation Unit
CP	Crude Protein	PL	Plantain
CRD	Completely Randomized Design	PL-D	Plantain Lemongrass Diet
CRP	C- Reactive protein	ppm	Parts per Million
DAB	Diaminobenzidine	PT-D	Plantain Diet

DCF	Digestible Crude Fiber	RBC	Red Blood Cell
DCP	Digestible Crude Protein	RBO	Rice bran oil
DDPB	Desirable Dietary Pattern for Bangladesh	RDI	Recommended Dietary Intake
DEE	Digestible Ether Extract	REA	Rib eye area
DM	Dry Matter	RSA	Radical Scavenging Activity
DMI	Dry Matter Intake	SD	Standard Deviation
DNFE	Digestible Nitrogen Free Extract	SDG	Strategic Development Goal
DOCs	Day Old Chicks	SE	Standard Error
DP	Dressing Percentage	SEM	Standard error mean
DPPH	2,2-Diphenyl-1-picrylhydrazyl	SFA	Saturated fatty acid
ED	Energy Density	SI	Egg shape index
EDRB	Energy Dense Rice Biscuit	SM	Spear Mi9nt
EDRC	Energy Dense Rice Cake	SME	Small and Medium Entrepreneurs
EE	Ether Extract	SOD	Superoxide dismutase
ELISA	Enzyme-Linked Immunosorbent Assay	SPSS	Statistical Package for Social Sciences
EPEF	European Production Efficiency Factor	SS	Stainless Steel
FA	Formaldehyde	T ₀	Formulated diet + AGP (Control)
FAO	Food and Agricultural Organization	T ₁	Formulated diet + 1.0 % (DM) Shade dried herb mixture
FCR	Feed Conversion Ratio	T ₂	Formulated diet + 1.0 % (DM) Blenching force dried herb mixture
Fe	Ferrous	TAC	Total Antioxidant capacity
FMD	Foot and mouth diseases	TAC	Total Anti-Oxidant Capacity
G	Gram	TC	Total Carotenoids
GB	Guobiao (Chinese) Standards	TDN	Total Digestible Nutrients
GC	Gas Chromatography	TLC	Thin Layer Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry	TLWG	Total Live Weight Gain
GL	Garlic	TMR	Total Mixed Ration
GoB	Government of Bangladesh	TS	Total Sugar
GPx	Glutathione Peroxidase	TSS	Total soluble solids
GQND	Grain Quality and Nutrition Division	TWC	Total White Cell
GR	Growth Rate	UK	United Kingdom
GT	Gelatinization Temperature	USA	United States of America
HDEP	Hen Day Egg Production	USFA	Unsaturated fatty acid
HDL	High Density Lipoprotein	UV	Ultraviolet
HDPE	High Density Polyethylene	VF	Vacuum Frying
HMP	Plantain, Ivy-Gourd, Mint and Garlic Powder	VP	Vacuum Pump
Hp	Horsepower	Vs	Versus
HPLC	High Performance Liquid Chromatography	WAC	Water Absorption Capacity
HSD	Honest Significant Difference	WB	World Bank
HU	Haught Unit	Zn	Zink

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Executive Summary

The sub-project with the general objective to control qualitative reduction/loss of food value and improvement of nutritional quality of rice, selected fruits/vegetable/herb products through value addition carried out research in three different selected areas. The component -1 (BRRI) of the sub-project worked to address malnutrition related problems of the vulnerable section of our population especially urban street children. Street children are generally malnourished due to their deprivation on health and improved nutrition. Our plan was to nourish children from malnourished. Since rice is an important source of energy, hypoallergenic, easily digested, providing protein with higher nutritional quality and has versatile functional nutraceutical properties so, rice based low cost balanced, nutritious and safe diet formulation will be able to address malnutrition related problems of the vulnerable section of our population especially urban street children. A total of 384 street children were subjected to study a baseline survey aged from 4 to 12 years old from 20 hot spots in Capital City, Dhaka. Among the respondents 63% were male and 37% were female from street children population of 384. In order to observe the recommended dietary intake (RDI) per day from 4 years to 12 years old street boys (n=243), we found 27 to 59% deficiency of RDI and 28 to 56% deficiency of RDI in our street girls (n=141). We have prepared Energy Dense Rice Biscuit (EDRB, 3.6% moisture, 515 kcal per 100 g of serving) and EDRC (5.0% moisture, 500 kcal per 100 g of serving). Since our Energy Dense Rice Cake (EDRC) has a potential of providing 500 kcal energy per 100 g serving so, we could predict that incorporating our improved rice-based product once a day along with their daily regular intake, it will be able to mitigate nutritional gap by 64 to 100% for street boys and noticeably 70 to 100% for girls. A total of 32 respondents were taken part in a four months period impact study on EDRC from street children population. Our data revealed that malnutrition related biochemical parameters specially CRP (decreased) and Prealbumin (increased) were significantly improved during four months supplementary intake of extra 500 kcal per 100 g serving of EDRC in tested street children's samples which resembles the possible impact of EDRC on street children. Rice-based bakery products specially EDRB and EDRC can potentially be used in school feeding nutritional program and disaster management in Bangladesh.

Component 2 (BARI) worked to development of technology on value added products with appropriate packaging of selected fruits and vegetables and simplification of selected processing equipment and optimization of operating parameters; In Bangladesh, fried chips are popular and common snack item for kids and middle-aged group people. But it may be dangerous for human health if any hazardous substances and carcinogenic compounds produce during frying. Deep oil frying at between 160° and 180°C is the most common practice that produces acrylamide. Vacuum frying is a novel method of producing high quality healthy fruits and vegetable chips. There is no information documented regarding the fried chips processing to marketing in our country. In this regard, the baseline survey was conducted at 08 locations viz Dhaka, Gazipur, Tangail, Mymensingh, Bogura, Munshigonj, Jashore and Khagrachari with a detailed survey questionnaire. Fifteen hawkers and five producers were randomly selected. All aged people in Bogura preferred fried potato chips (100%) but in Jashore (80%) kids liked fried potato chips. Fried chips processors used deep oil frying technique and most of them were unknown about healthiness knowledge of fried chips consumption. A simplified low cost and small scale vacuum frying machine was designed and fabricated with the help of local manufacturer. The fabricated vacuum frying machine comprises of three basic parts such as frying unit, condenser unit and vacuum pump. Frying unit belongs to frying basket (300 × 210) mm and two heating coils (4 KW, 220V, AC). Two-stage oil type vacuum pump of 1 hp was used to create low pressure in the frying chamber during chips frying. To release the excess oil from fried chips, a de-oiling machine was also designed and locally fabricated. The machine comprised of two basic parts such as de-oiling chamber and control panel. The capacity of de-oiling machine is 600-800 g/batch. A centrifugal motor of 1.5 hp was attached to rotate the basket. The overall fabricated cost of vacuum fryer with de-oiling machine is about 1.50-1.70 lakh Tk. The developed machine will be beneficial to the SME level for producing quality fried chips. A series of experiments was conducted to optimize the processing parameters for development of vacuum fried chips products from banana, sweet potato, jackfruit etc. Banana chips were developed to optimize the combination of frying temperature-time and to evaluate shelf life with nutritional quality during storage. Unripe, matured with uniform size of tissue cultured BARI Kola-1 was selected and thinly sliced (3-4 mm). Raw slices were soaked into lemon and salt mixed water solution and then again soaked into 5% turmeric solution for 2-3 hours. Three

frying temperature (110, 120 & 130°C) and three frying time (8, 10 & 12 min) were considered. Banana chips fried using BARI developed vacuum fryer at 120°C for 12 minutes exhibited best sensory score initially 8.2 and after 06 months of storage HDPE packet. Similarly, BARI Sweet Potato-7 variety was selected for vacuum fried sweet potato chips. In case of consumer preference test the highest overall acceptability, score was observed by expert panelists in treatment T₆ (6.90) (110°C & 12 min). The sensory score indicated that T₆ retained more sensory attributes among the treatments. In case of jackfruit chips, matured Khaja type jackfruit was selected and then peeled. Jackfruit bulbs were sliced and fried at 03 frying temperature and 05 frying times. Jackfruit chips fried at 110°C for 25 minutes and 120°C for 20 minutes exhibited best in terms of nutritional quality and shelf life during 06 months of storage in HDPE packet. BARI developed low-cost vacuum fryer with de-oiling machine and a package of processing protocol will add value in agro-processing industry for producing quality fried chips products from agricultural produces which will be safe for human consumption and will create income generation for the people in the country.

Component 3 (BAU) worked for profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh and *in-vivo* trail on poultry, sheep, and dairy cattle to find out performance potentials, correct threshold level, digestion and absorption dynamics and % of PCs retain in the meat, milk and egg etc. Use of feed grade antibiotics had been a common practice in animal production over the past 4-5 decades. The use of antibiotics as feed additives is hazardous due to cross-resistance and multiple resistances of pathogens. So, to produce safe animal products, use of different herbal plants could be a solution. Use of antibiotic growth promoter in livestock production keep some residual effects in their products but herbal supplementation doesn't have any hazardous affects both livestock and human health. For these reasons this study was undertaken to identify different available medicinal herbs in our country which have growth enhancing capability and will enhance the production of safe livestock products. The proposed study were executed through the detection of plant phytochemicals such as organic phenol, flavonoids, saponin, tannin, carotenoids contents from different herbs that are locally available. *In-vivo* trail was performed in dairy cows, beef cattle, sheep and poultry to find actual performance potential, determination of correct threshold level, digestion and absorption dynamics by metabolic trail, and plasma biomolecules profile. An On-Station sheep feeding trial was performed using solely plantain or garlic leaves and their mix that enhance growth performance, serum lipid profiles, serum antioxidants status, immune status, meat quality and carcass characteristic in lambs. An On-Station broiler feeding trial was performed using plantain, spearmint, garlic and ivy-gourd mix powder at different forms for observing growth performance, meat nutritional value, and shank mineralization. The shade dried herbs mixture form are suitable for improving the production performance and meat quality of broilers. An On-Farm layer feeding trial was performed using plantain, ivy-gourd, garlic, and spearmint mix at 0, 5, 10, 15 and 20g/kg feed to evaluate the laying hen performance, egg external and internal quality. The herbs mixture at 1.0-1.5 g/kg diet might be used for the increased of egg production, and egg external and internal quality. A dairy cattle trial was performed using different level of Plantain herb (*Plantago lanceolata* L.) herb powder (PHP) at 0, 50, 100 and 150 g/animal. A total of 12 Holstein Friesian crossbred cows were randomly allocated for a period of 63 days to assess the milk production performance and milk quality in dairy cows. The milk yield, composition, DPPH, beta carotene, milk immunoglobulin's level were obtained better at 100g plantain supplementation per animal per day. A mouse model (Validation trail) was performed using plant bioactive components acteoside and synthetic acteoside at different level in albino mouse (*Mus musculus*). Plant bioactive components acteoside positively improved the serum antioxidant status that reduced the production of free radical thus resulting increased growth. Besides, acteoside plant bioactive components improved the liver enzymatic activity and liver health. The health of gastrointestinal tract was improved in mouse by herbal bioactive components. So, plantain herb is safe and acts as growth promoter for livestock that enhanced milk, meat and egg production which are enriched with antioxidants and safe for human consumption.

Keywords: Energy dense rice biscuit, Formulated food, BARI vacuum frying machine, Quality fried chips, Medicinal herbs, Phytochemicals.

PBRG Sub-project Completion Report (PCR)

A. Sub-project Description

1. Title of the PBRG sub-project

Fortification and Standardization of Nutritional Level in Selected Human Foods and Efficacy Test of Polyphenolic Compounds in Livestock Products

2. Implementing organizations

Bangladesh Agricultural Research Council, Bangladesh Rice Research Institute, Bangladesh Agricultural Research Institute and Bangladesh Agricultural University

3. Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator, PI/Co-PI (s)

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4. Sub-project budget (Tk.)

4.1. Total (in Taka. as approved) : 2,53,53,000.00 (Two Crore Seventy Five Lac Taka)

A. Coordination component	: Tk. 1,86,000.00
B. Component 1 (BRRI)	: Tk. 62,78,000.00
C. Component 2 (BARI)	: Tk. 87,15,000.00
D. Component 4 (BAU)	: Tk. 85,00,000.00

4.2. Latest revised (if any) : Not applicable

5. Duration of the sub-project

5.1 Start date : 14 August 2018

5.2 End date : 31 December 2021

6. Background of the sub-project

In a street children consortium on promoting and protecting the rights of street children at Colombo, Srilanka back in 2001, Aparajeyo Bangladesh, for the first time bring the statistics that the estimated number of street children was 445226 in Bangladesh of which 75% were in Dhaka city and frequency were 53% boys and 47% girls (AUEssays, 2018). Bangladesh Institute of Development Studies (BIDS) had reported in December 2004, a total number of street children in Bangladesh was 6, 74,178 (Choudhury and Juliano, 1980). In 2015, according to BIDS, the number becomes 1.5 million and it will reach to 1.56 million in 2024 (CDC, 2015). Rural-urban migration is one of the major reasons of the exposure of the street children to the vulnerabilities. Considering the increasing trend of street children and diversity of the vulnerabilities, it is very much necessary to take proper and effective steps to protect the children from all types of violations, to ensure their basic necessities properly, to ensure their well growth and development. The given situation clearly reveals that there are no comprehensive and reliable statistics available on the actual numbers, living conditions, needs and interests of children living on the streets. But the fact is street children constitute one of the most vulnerable and marginal groups in Bangladesh. Hakim and Rahman (2016) in a study on health and nutritional condition of street children of Dhaka City revealed that about 65% street children are under weight and 22.5% children eat only two times in a day (DDPB, 2013). Masud et al. 2011 conducted a population-based survey on lives and livelihoods on the streets of Dhaka City and his data revealed that housing, food, and lack of jobs are the three most common problems for which street people are commonly sought assistance (FAO, 2004). Although there are several reports, case studies, surveys are available on street children of Bangladesh but very little information are available regarding food formulation especially for their nutritional requirement to address malnutrition. Thus, the aim of the research is to intervent at this point to make a concrete step to eradicate malnutrition with formulated rice-based food items. Since rice is an important source of energy, hypoallergenic, easily digested, providing protein with higher nutritional quality and has versatile functional nutraceutical properties (Shozib *et al.*, 2018) so, rice based low cost balanced, nutritious and safe diet formulation will able to address malnutrition related problems of the vulnerable section of our population especially urban street children. Among cereals, rice do not have gluten protein. Since gluten protein of wheat and maize cereals produce hypersensitivity reaction to human and we do not have multiple opinions other than consuming rice only so, rice-based food items such as rice cookies, rice ball, rice cake, rice noodles can be formulated at lower cost but higher nutritious value. We were aimed to formulate low cost and nutritionally balanced rice-based food items from nutritionally enriched selected brown and pre-germinated brown rice varieties. Finally, we had a plan to operate a volunteer promotion scheme of rice-based food items with multiple options for the malnourished children in urban city Dhaka. The small amount of quality rice-based products intake will reduce the consumption of rice intake gradually in Bangladesh. Street children problem in Bangladesh are social phenomena which also be given importance to enhance social and economic justice and equity. Our population target is children at age of 4 to12 years and their daily recommended dietary intake (RDI in kcal of energy) requirements of boys and girls (from 4 years to 12 years of age) ranges from 1303-2200 and 1202-2070 kcal/day according to FAO for Bangladeshi population (Hakim and Rahman, 2016; Mohammad, 2009). Rice based formulated diets for street children with moderate to acute malnutrition must have some important characteristics including high content of micronutrients, especially growth (Type II)

nutrients, high energy density, adequate high protein and fat content, low content of antinutrients, low risk of contamination, acceptable taste and texture, culturally acceptable, easy to prepare, affordable and available. We tried to formulate our rice-based products with several ingredients including rice, egg, rice bran oil (RBO), salt, sugar etc. We had to focus on energy density of formulated food. If the energy density is too low, the food becomes too bulky, and the children will not be able to eat adequate amounts. We have a target to formulate food which will supplement at least 30% of total energy requirement by one meal solution and the formulated food should be high energy density (ED) food. The energy density is most important for children with wasting, as they have an increased energy need for catch-up growth.

In addition to that increasing demand of low cost high nutrient rich safe food product from various underutilized seasonal fruits and vegetables has become a national need. At present chips prepared from different fruits and vegetables are well known and popular snack food in Bangladesh. Children are the potential consumers of it. However, chips are equally liked by people of different ages. Most popular chips available in the market are real potato chips and potato crackers. The first one is prepared directly from sliced potatoes and the second one is from dough mixture. Chips prepared from seasonal fruits are hardly available in our market except banana chips. Enterprises based on seasonal fruit chips are advantageous from many corners. It is well-known that fruits and vegetables are seasonal and location specific. In the harvesting period, they are available in plenty and do not fetch a fair price for the producers, while in other seasons they are not available at any price. These constraints result in huge postharvest loss of agro-produces every year in the country (~40%) and the producers face economic loss every year. Jack-fruit, pineapple, mango, guava and papaya are some of the seasonal fruits which are susceptible to huge post-harvest loss. If they are processed into high quality popular snack products like chips, their postharvest loss will be minimized, farmers will get fair price, production of them will be increased and finally food and nutrition security of producers and consumers will be obtained.

Secondly fresh fruits and vegetables are rich in carbohydrates, fibre and vitamins. They are essential for human body. It is known that soluble fibre improves gut environment, which reduces the risk of chronic diseases. Antioxidant and phenolic compounds essential part of fruits and vegetables are also essential for reducing risk of cardiovascular disease. Hence, processing of fresh produces into chips will be advantageous from nutritional point of view as well.

Thirdly, traditional deep fat oil frying results in low quality chips in terms of colour especially from high sugar fruits (banana chips as an example). Deep frying develops deep brown and blackish colour. Deep brown and blackish colours are developed due to caramelization and non-enzymatic browning of starchy materials. Colour thus formed is associated with acrylamide, a potential carcinogenic for human health found in deep fat fried products. This harness can easily be overcome by employing vacuum frying technology. By this method, frying will be carried out under vacuum pressure at low temperature and hence, product quality will be improved, nutrition loss will be minimized, degree of fried oil deterioration will be reduced and finally processing cost will be minimized.

In Bangladesh, antibiotic growth promoters (AGPs) were introduced to livestock industry to improve the animal productivity but in 2006 European Union banned the AGPs in livestock feed

industry due to its negative consequence in human health that cause anti-microbial resistance (AMR). Therefore, as an alternative to AGPs, prebiotic, probiotic, enzymes and herbals additive have recently gained much attention, whereas herbal additive have been examined to be safe, economic, and less hazardous among all feed additives in livestock industry (Qureshi *et al.*, 2016). Herbal additive refers to natural herbs, spices and their extract that used as non-nutritive products in ruminants and non-ruminant's nutrition to elevate the performance, and products quality especially milk, meat and egg antioxidants level (Windisch *et al.*, 2008). Herbal supplementation improved feed utilization by increasing the secretion of enzymes from digestive tract and liver (Hernandez *et al.*, 2004; Prakash and Srinivasan, 2010; Abou-Elkhair *et al.*, 2014) due to its antioxidants, antimicrobial and anti-inflammatory activities (Windisch *et al.*, 2008; Gheisar and Kim, 2017).

Asia including Bangladesh is treasurer of medicinal herbs. Medicinal herbs have been used as human tonic, and forage herbs for sheep, broiler, layer and dairy production (Fons *et al.*, 2008; Camy *et al.*, 2019; Redoy *et al.*, 2020) which exerts anti-microbial, anti-oxidative, anti-inflammatory, anti-parasitic effects in livestock especially broiler, layer, sheep, and dairy cattle (Hausmann *et al.*, 2007; Ferrazzano *et al.*, 2015; Boamah *et al.*, 2016), due to its bioactive components. In addition, medicinal herbs have higher capability to scavenge the free radicals (Al-Mamun *et al.*, 2007) that might reduce the oxidation leading to raise the milk, meat and egg quality. Moreover, medicinal forage herbs are rich source of flavonoids, glycosides, phenols, alkaloids, terpenoids and tannins that showed the antioxidants properties (Ashwini *et al.*, 2012), anti-inflammatory activity (Deshpande *et al.*, 2011), and hepato-protective activity (Kumar, A. 2012). Medicinal herbs are aromatic in nature due to its volatile components. So, the available medicinal herbs might be used in safe and quality livestock production including sheep, broiler, layer and dairy cattle. But the threshold level is an important factor as herbs possesses phytochemicals like polyphenols, glycosides, tannins, and saponins which have positive impact on livestock along with human health but overdose might lead to reduce feed intake, nutrients absorption, bone formation and poisoning not only in livestock (Ebrahim *et al.*, 2015) but also in human (Lorent *et al.*, 2014). So the research was designed for profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh and to find out performance potentials, and validation of specific effect of PCs in both human and animal body.

7. Sub-project general objectives

To control qualitative reduction/loss of food value and improvement of nutritional quality of rice, selected fruits/vegetable/herb products through value addition.

8. Sub-project specific objectives

8.1. Coordination component (BARC) : To ensure smooth and efficient implementation of sub-project activities to achieve desired sub-project outputs within the stipulated timeframe under strengthened capable research management system.

- i. To coordinate sub-project implementation efforts and integration of activities to generate desired information /technology as per methodology of the sub-projects;
- ii. Identify operational deviations and addressing constraints/problems (if any) under a process of strong and regular monitoring of the project activities.

- iii. To upgrading the level of output of the sub-project through reviewing of yearly technical progress.
- iv. Collect and collate sub-project data, finding and observation and production of compiled Project Completion (PCR).

8.2. Component-1 (BRRI)

- i. Survey on dietary pattern of street children in capital city, Dhaka.
- ii. Formulation of low-cost rice-based nutraceutical food items.
- iii. Impact evaluation studies of formulated rice-based foods.

8.3. Component-2 (BARI)

- i. Simplification of selected processing equipment (vacuum fryer) and optimization of its operating parameters.
- ii. Technology development on value added products (fruit/vegetable chips) of selected fruits and vegetables;
- iii. Enhancement/fortification in selected food products (fruit/vegetable chips) by adding selected vitamins, minerals and extracted suitable plant pigments.

8.4. Component-3 (BAU)

- i. Profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh.
- ii. *In-vivo* trial on poultry, sheep, and dairy cattle to find out performance potentials, correct threshold level, digestion and absorption dynamics and % of PCs retain in the meat, milk and egg.
- iii. Validation of specific effect of PCs in both human and animal body through different mouse model.

9. Implementing location (s)

9.1. Component-1 (BRRI) : BRRI, Gazipur and Dhaka Metro

9.2. Component-2 (BARI) : Dhaka, Gazipur, Tangail, Mymensingh, Chattogram, Khagrachori, Jashore, Bogura

9.3. Component-3 (BAU) : Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh-2202

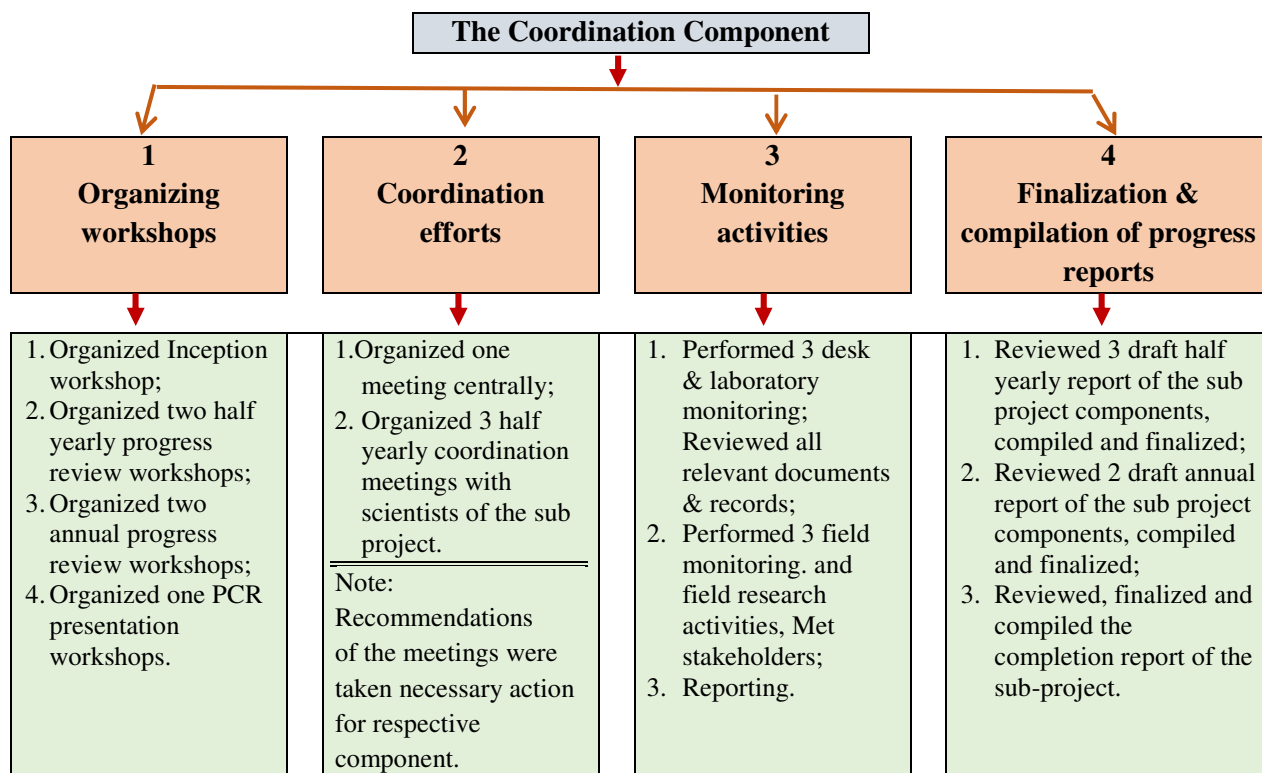
10. Methodology in brief

10.1. Activities implementation approach of 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 projects 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 sub-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. Following are the major activities carried out by the Coordination component under the plan:

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

The implementation approach and activities thereunder 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 presenting 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 October' 2018	Attended all PI, CoPI & expert members.
Revision of PP	Done as per recommendations of Inception workshop	
Half yearlyprog. Review Workshop (Date)	Organized centrally at BARC in March' 2019, January' 2020.	Attended all PI, CoPI & expert members
Ann. Prog. review Workshop (Date)	Organized centrally at BARC in June' 2019 & in September' 2020	Attended all PI, CoPI & expert members.
Coordination meeting (No)	03 24.10.19, 19.02.20 & 18.06.20	One Coordination meeting held centrally.
Monitoring of field and Lab activities	04 (BIRRI, BARI & BAU)	Covered all components under sub-project.
Financial achievement	100% of released money & 99.29% of total approved budget	-
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



Centrally organized inception workshop at BARC



First Annual progress review workshop of PBRG sub-projects



Half yearly progress review workshop of PBRG sub-projects



Second annual progress review workshop of PBRG sub-projects



Field visit by the Coordinator at BRRI and BARI



10.2. Research methodology (*Combined*)

10.2.1 Baseline survey on street children in capital city Dhaka

Street children are the vulnerable group of children coming to fight to gild the streets for their habitual abode and livelihood drifted into a nomadic life. These children are generally malnourished due to their deprivation on health and improved nutrition. Our plan was to nourished children from malnourished. To plan a need-based information is essential on their dietary pattern of street children. A baseline survey was done by the component -1 of the sub-project on 384 samples of the target population (street children in Dhaka city) to fill in this knowledge gap.

10.2.1.1 Study areas

A total of 20 study areas including all previously reported hot spot areas of street children in Dhaka City were accounted for conducting the baseline survey such as Shahbagh (Poribagh and shishu park area), Ramna (Dhaka University promises), Karwan Bazar, Kamlapur (Rail station area), Tejgoan (Industrial area), Agargoan (IDB Bhabon to Shamoli shishumela), Azimpur (Newmarket and graveyard), Baily road area, Shadorghat (Terminal area), Gulistan (Stadium area), Syedabad (Bus station area), Khilgoan (nearby Flyover area), Muhammadpur (Market and bus stand area), Mohakhali (Tangail bus stand to ICDDR area), Mirpur 2 (Stadium area), Gabtoli (Bus station area to technicalmore), Uttara (Jasimuddin to house building area), Gulshan (circle 1 to circle 2 area), Moddo Badda (link road area), Rampura (Aftabnagor and Bonosri area).

10.2.1.2 Target population

Since street children are the most vulnerable group of children coming to fight to gild the streets for their habitual abode and livelihood drifted into a nomadic life. These children are generally malnourished due to their deprivation on health and improved nutrition. Our target population is both boys and girls of age from 4-12 years old street children.

10.2.1.3 Sampling and sample size

Interviewed 384 street children from above mentioned 20 hot spots. Using Cochran Equation, we find the sample size

$$\text{sample size} = \frac{z^2 P (1 - P)}{E^2}$$

The value of z is found in statistical tables which is contain the area under the normal curve. $z = 1.96$ for 95% level of confidence. P is the estimated proportion of an attribute that is present in the population. E^2 is the desired level of precision.

$$\text{Sample size} = \frac{1.96^2 \times 0.5 \times (1 - 0.5)}{0.05^2} = 384.16 \cong 384$$

10.2.1.4 Development of tools

A semi-structured questionnaire was developed to collect data through face-to-face interview with the respondents. The questionnaire was pre-tested in areas outside our sample and were revised on the basis of feedback received from field-testing. The quantitative questionnaire mainly covered diverse information of study respondents such as socioeconomic and demographic characteristics, current living conditions and livelihood activities, morbidity and health-seeking behavior, dietary intake pattern, and education and drug uses, and anthropometrical measurement such as BW and height, BMI, waist and hip circumferences ratio, mid-arm circumference. Since the aim was to focus mainly in daily energy intake of street children in our baseline survey so we considered their calorie information for this study.

10.2.1.5. The survey

An instruction manual explaining the key terms in the questionnaire was developed and given to the enumerators. On the day of interview, the team identified the first respondent according to the definition of street dwelling child population and criteria for selection. The study included only those who will show interests on volunteer basis. Verbal consent will be taken before starting the interview. The field activities were supervised by the authors. Quality controls were measured accordingly. Before launching the actual study, all the techniques and tools were tested and modified under pre-testing. Enumerators were given extensive training, both in the classroom and in the field setting until the researchers were convinced about their abilities in collecting and managing data effectively and consistently. A field guideline detailing the definitions used, identification of prospective respondents, the steps to be followed during data collection, and trouble-shooting measures will be prepared for the data collectors. Effective and supportive supervision should ensure on a continuous basis. The investigators supervised the data collection and verified the validity, accuracy and completeness of data through on-the-spot checking. Regular feedbacks were given to the enumerators for maintaining an acceptable level of data quality. Regarding ethical issues, all respondents were informed about the purpose of the study and their verbal consent will obtained before taking interviews. All information will be kept anonymous and confidential. Data management and analysis: The questionnaires were checked in the field by the supervisors for any inconsistency and incompleteness.

10.3.1 Questionnaire development and conducting the baseline survey of existing fried chips products from processing to marketing

A detailed survey questionnaire was prepared by component 2 (BARI) with the assistance of the Agricultural Economics Division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The questionnaire was segmented into two types (Based on Hawkers and based on Producer) to facilitate the data collection and to get the entire information from the selected location. Then, it was pre-tested in the selected project areas. The baseline information was collected from seven districts namely Dhaka, Gazipur, Tangail, Mymensingh, Bogura, Munshigonj and Jashore. Fifteen hawkers and five producers were randomly selected from each location to collect the information by the selected questionnaire. During the survey the complete data from producers to consumers was collected (Tables 11-13). The information on selling pattern viz., selling area, retail price, profit, selling time etc. were collected. The processing

pattern with their frying practice parameters such as frying technique, frying oil type, frying temperature & time, raw materials, packaging, storage etc. were recorded during the survey. Both hawkers and producers based questionnaire covers all qualitative and quantitative data, which covers both processing methods and marketing channel with their profit and popularity group of the consumers.

10.3.2 Data collection and data analysis

The collected information was organized in tabular form by using MS Excel data sheet and then analyzed. MS Excel was used for statistical analysis, which provided different information from fried potato chips processing to marketing status. The information also represented different comparative study among the parameters.

10.4.1. Formulation of rice-based energy dense bakery food items:

Our population target is children at age of 4 to 12 years and their expected weight ranges from 16.71 to 34.38 kg for boys and 16.0 to 37.0 kg for girls as per Bangladesh population standard including both sexes (Hakim and Rahman, 2016). So formulation of rice based our rice-based products done with several ingredients including rice flour (selective), sugar, skimmed milk, egg, vanilla essence, grinded sagu powder and extract of aloe vera as lubricating agent, butter, yeast powder, Rice Bran Oil (RBO) and vegetable edible oil. The metabolizable energy values of all formulated foods ingredient was given in kilocalories (kcal). Thus the energy values have to be calculated based on protein, fat, available carbohydrates and fibre values and by applying the energy conversion factors such as kcal/g 4, 9, 4 and 2 Kcal respectively. Since rice flour does not have gluten protein unlike wheat, so making dough was seeming difficult to get appropriate texture in reality. We had tried to overcome the problem with few measurements such as un-parboiled milled rice were treated for one hour soaking in water at room temperature before grinding into flour, Both low Apparent Amylose Content (AAC) and Gelatinization Temperature (GT) rice flour were mostly suitable for rice-based bakery products, Flour parameters such as Oil Absorption Capacity (OAC), Water Absorption Capacity (WAC) and forming activity etc. would be considered at flour quality selection, Sheaving flour to attain unique flour size is a necessary step and Sagu, casava and aloe vera can potentially be used as thickening, stabilizing, suspending, and binding agent.

10.4.1.1. Proximate analysis of energy dense rice-based bakery product

Estimation of Protein : Standard micro Kjeldahl procedure of AOAC (Mohammed and Mahfuja, 2016) were used for the determination of nitrogen and crude protein were estimated by multiplying the nitrogen content by a factor 5.95 for rice flour and 6.25 for egg.

Moisture : Moisture content were determined by AOAC method (Mohammed and Mahfuja, 2016).

Dietary fibre (g)/crude fibre : Dietary fiber were determined by AOAC method 2000 (NIST, SRM 1568b).

Ash (g) : Ash content of grain were estimated by heating the dried raw sample in a Muffle furnace at 600°C to burn out all organic materials for 3-5 hours till to constant weight. The inorganic residue will be quantified as the ash content by AOAC method 2000 (Procedure, 979.09).

Estimation of Fat : Fat were extracted from the grounded rice samples with Chloroform: Methanol (2:1) solution. Fat was determined from the extract by the method of Choudhury and Juliano (1980).

Carbohydrate (g) : The carbohydrate content of a sample was calculated by subtracting the percentage of other components of that sample (moisture, ash, fat, protein and fiber) from 100. Percentage of carbohydrate = 100 - (moisture + ash + fat + protein + fiber).

Estimation of Minerals and Heavy metals : Minerals (Zn, Fe, Ca) and heavy metals (As, Pb, Ni and Cd) were estimated in AAS by method of the Association of Official Agricultural Chemists (AOAC 2020; procedure, 942.05). National Institute of Standards and Technology (NIST) reference material Rice flour NIST SRM 1568b SIGMA-ALDRICH, USA was used for mineral and heavy metal estimation in Atomic Absorption Spectrophotometer (AAS) (AOAC, 2019, 21st edition). Estimation of phosphorus was carried out by measuring calorimetrically the blue color formed when the ash solution was treated with ammonium molybdate and the phosphomolybdate thus formed was reduced (Syed 2011).

10.4.1.2. Blood chemistry

CBC (Complete Blood Count) including Hb%, TWC (Total White cell count), RBC (Red Blood Cell Count) were analyzed by automated hematology analyzer (Brand: Landmark Biomedicals). Serum levels also decline in patients with conditions associated with protein malnutrition, such as malignancy, cirrhosis, protein-losing enteropathy, and zinc deficiency. Serum Prealbumin was estimated by Human Prealbumin ELISA Kit (ab231920) (Yoshida *et al.*, 1976).

10.4.1.3. Impact evaluation study on energy dense rice food product on street children

Formulated foods such as EDRC and EDRB were prepared based on the calorie gap between recommended calorie intake and actually calorie intake for the age 4 to 12 years for both sexes. Regarding impact evaluation we have divided our required samples (Total 52 samples; 32 samples for treatment: 20 for control into two groups. Treatment groups (having formulated rice-based foods for four months' time) and control group (without formulated rice-based foods). These two groups were selected using propensity score matching. Test group children used to gather in an open school for spending one hour time while intaking the supplied EDRC and their activities were routinely digitally monitored. Then mean difference in different parameters were analyzed by independent t test and paired t-test in R version 4.02 and we were able to come to a decision whether this special supplementary diet can improve present status of the street children or not.

10.5.1. Fabrication of vacuum frying machine

A small scale and low cost vacuum frying machine was designed and fabricated with the help of local workshop by the Postharvest Technology Division (PHTD) of BARI, Gazipur. A number of factors were considered in the design of the vacuum frying machine which include the physical, mechanical properties and availability of the spare parts for construction and repairing easily by the local workshop. Other considerations in designing of the frying machine was to make it easy to operate and feasible in transportation and installment. The designed vacuum fryer was drawn with Solidworks 16 software. The vacuum fryer was consisted of three different units of vacuum frying unit, condenser unit and vacuum pump. Each unit was composed of different spare parts. The functional major parts in detailed description following:

Vacuum frying unit : The vacuum frying unit/chamber is an airtight vessel (360×412 mm) made by aluminum sheet where the frying of the pre-treated raw materials was conducted. The vessel was set over a base of SS bar (860×390×40 mm) and connected with the control panel (300×300×180 mm). During frying, the vessel needs to fill by 15-20 Liter vegetable oil. Inside the vessel two electric heating coil (4000 Watt, 220V, AC) were set at the bottom crossly and a sample holding basket (300×210 mm). Sample holder contained a handle (180×3 mm) to facilitate the operations. It holds pores of 10 mm. The schematic diagram of the sample holder was shown in the Fig 1. A temperature probe inserted in the middle of the frying oil which is connected with the thermocouple of the control panel, which monitored the desired temperature (0-400°C) of the oil and heater on/off condition. A pressure gauge range 0 to -760 mmHg was set at the lid of the vessel. A one-way stainless-steel ball valve was inserted at the top of the vessel to pull out the produced steam during frying of the products. At the lower portion a discharge option for frying oil made available by one way ball valve, which facilitates the collection of the fried oil after frying and cleaning the vessel. Four nylon wheels were also attached with the base to make easy to move from one place to another.

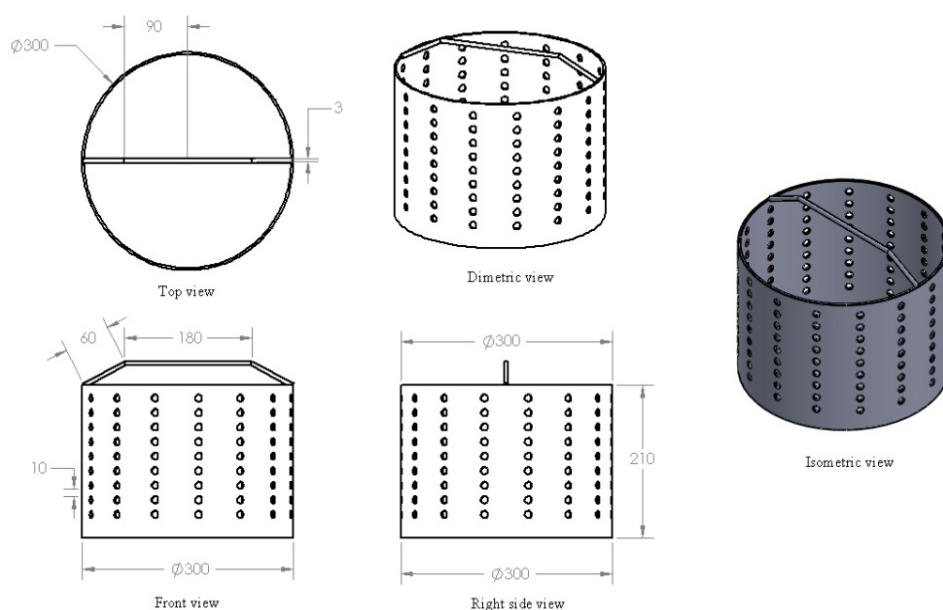


Fig 1. Schematic view of the sample holder for the vacuum frying unit.

Control Panel box : To visualize the heater and vacuum pump on/off condition different indicator light and digital temperature controller was attached in the control panel. An emergency switch was also incorporated in the control panel to stop instantly the machine avoiding any unexpected situation like operational misleading, mechanical fault, electricity failure etc.

Condenser Unit : A condenser is an important part of the vacuum frying machine that was provided to trap the evolved steam during frying by condensation. Stainless steel made condenser unit (520×165 mm) was supported by three legs (256×32 mm). The heat exchanged in the condenser unit with vertical cooling tubes (30 no. copper tubes) (380×10 mm). The cooled water passed continuously surrounding the tubes. The apex of the condenser unit was connected with frying chamber through the heat proof pipe in the gate valve inserted at the top of the frying vessel. At the lower portion of the cooling tubes of the condenser also attached with the vacuum pump (1 Hp) through heat stable pipe to pull out the generated steam in the frying chamber. Ultimately, the produced steam passed from vessel to the vacuum pump through condenser unit. In the bottom of the condenser, there was a stainless steel one way gate valve to discharge the condensed steam. The schematic views of the condenser parts shown in Fig 2.

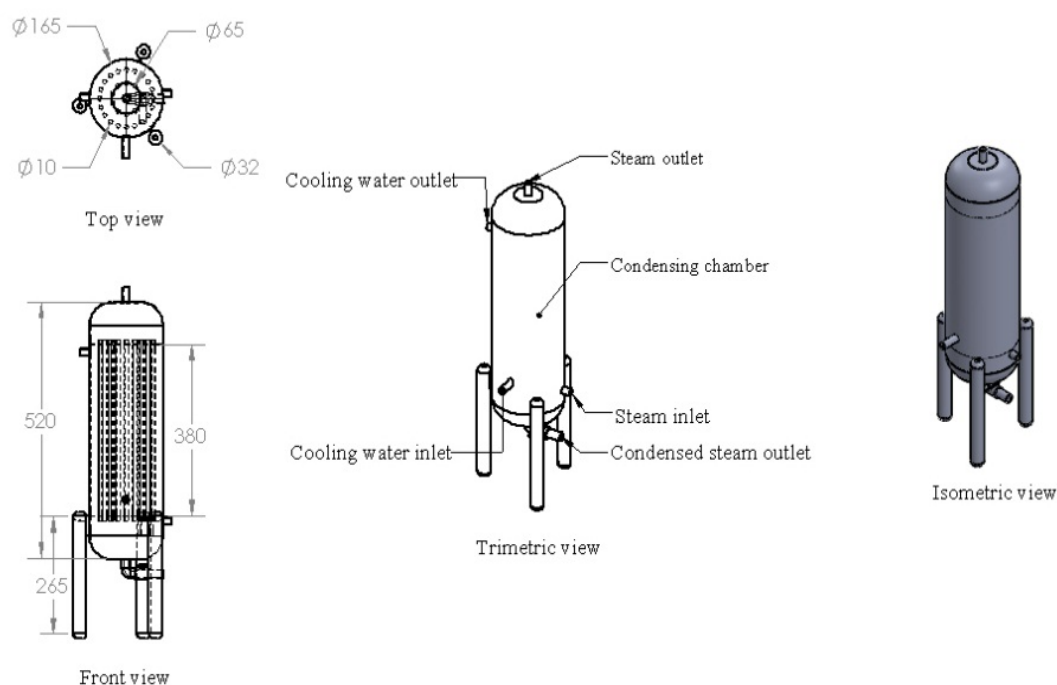


Fig 2. Schematic view of the condenser unit for the vacuum fryer.

Vacuum pump : Vacuum pump attached to lower the pressure below atmospheric pressure of the frying chamber. Two-stage vacuum pump (1 Hp) (Free air displacement: 9 CFM) (Model: VP 280) (used to get the optimum pressures for the process and discharging the steam generated during frying. The vacuum creation capacity possibly increased with increasing of the pump capacity. Oil type vacuum pump used for vacuum fryer. After several times running, it is required to change the oil of the vacuum pump.

10.5.2. Fabrication of de-oiling machine

A small scale and low-cost de-oiling machine was designed and constructed with the help of local workshop by the PHTD of BARI, Gazipur. The machine was named as BARI de-oiling machine. A number of factors were considered in designing of the de-oiling machine which include the physical, mechanical properties and availability of the spare parts for feasible construction by the local workshop. Other considerations in designing of the frying machine was to make it easy to operate and easily transportable and installation.

The designed and fabricated de-oiling machine basically consists of the de-oiling chamber and control panel. Each unit composed of different spare parts. The sketches of the designed de-oiling machine were drawn with solidworks software to visualize the measurements of the equipment. The functional properties and the dimensions of all the major parts are described individually in details:

De-oiling chamber : The de-oiling chamber was comprised of a longitudinal perforated stainless steel made basket (240×230) where the fried products are kept for de-oiling. The basket was set with a shaft and enclosed the chamber (300×290) with plain stainless-steel sheet, which was set over the base (560×360×350). To rotate the basket clockwise with high speed the basket shaft (30×100×25) connected with a centrifugal motor (180×160×140) of which capacity was 1.5 (Hp). The de-oiling basket was vertical cylindrical in shape of which is 230 mm dia and 240 mm height (Fig 3). It is made of perforated stainless steel sheet folding. The sheet composed of the 8 mm diameter perforation at 10 mm distance. But the lower portion was made of a plain heavy stainless steel sheet which was attached with the shaft of the pulley connected with the shaft of the motor by rubber belt. The de-oiling basket was covered by a plain stainless sheet (290×400) mm to cover up (Fig 4) and at the lower portion a de-oiled oil collection line of (250×20×30) mm was also attached to facilitate the reusing of the collected oil. To increase the rotation speed of the basket the 127 mm dia puli was used by rubber belt with the motor shaft. On the top of the de-oiling chamber a closed lid dia 300 mm with a handle (100×20) of stainless steel was set to prevent the noise during de-oiling. To de-oil the products the fried products were kept inside and then close the lid before starting the motor.

Control panel : A control panel board was fixed in the base of the de-oiling machine. A digital time controller (seconds/minutes/hours can be set) was connected in the control box where the de-oiling can be fixed and automatically stopped after desired time de-oiling. Motor on/off indicator light and alarming system also attached in the control box to visualize of the process de-oiling completion. An emergency switch also attached to stop the process in an emergency case to prevent any unwanted situation.

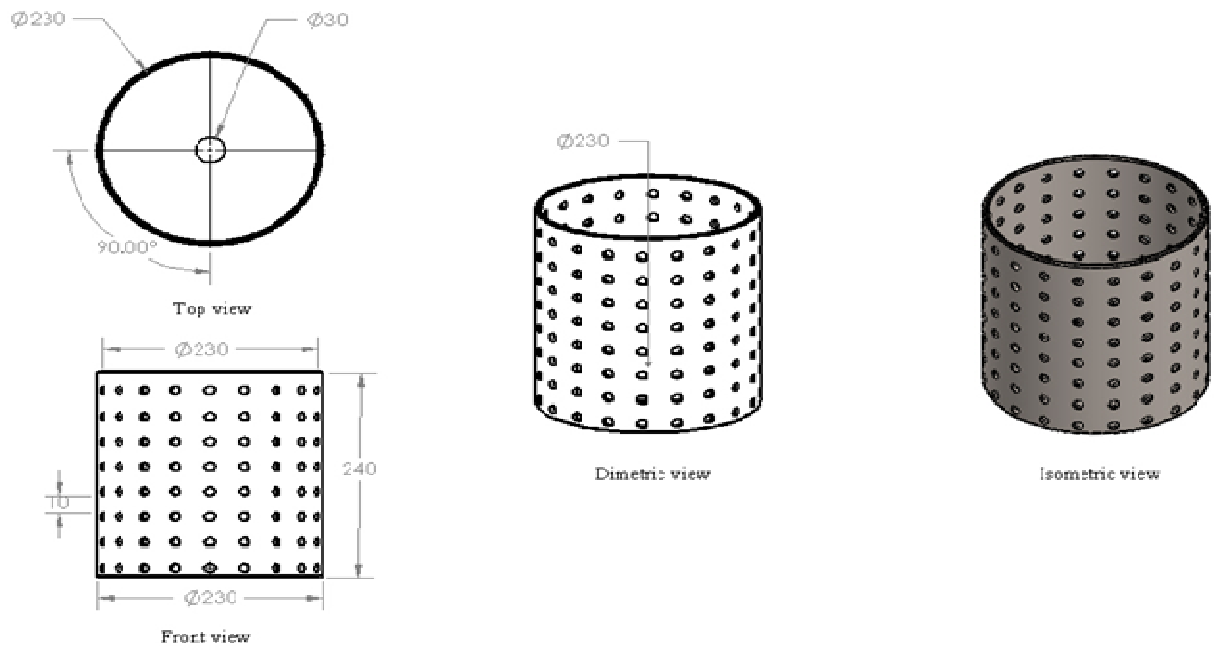


Fig 3. Top, front, dimetric and isometric views of de-oiling basket.

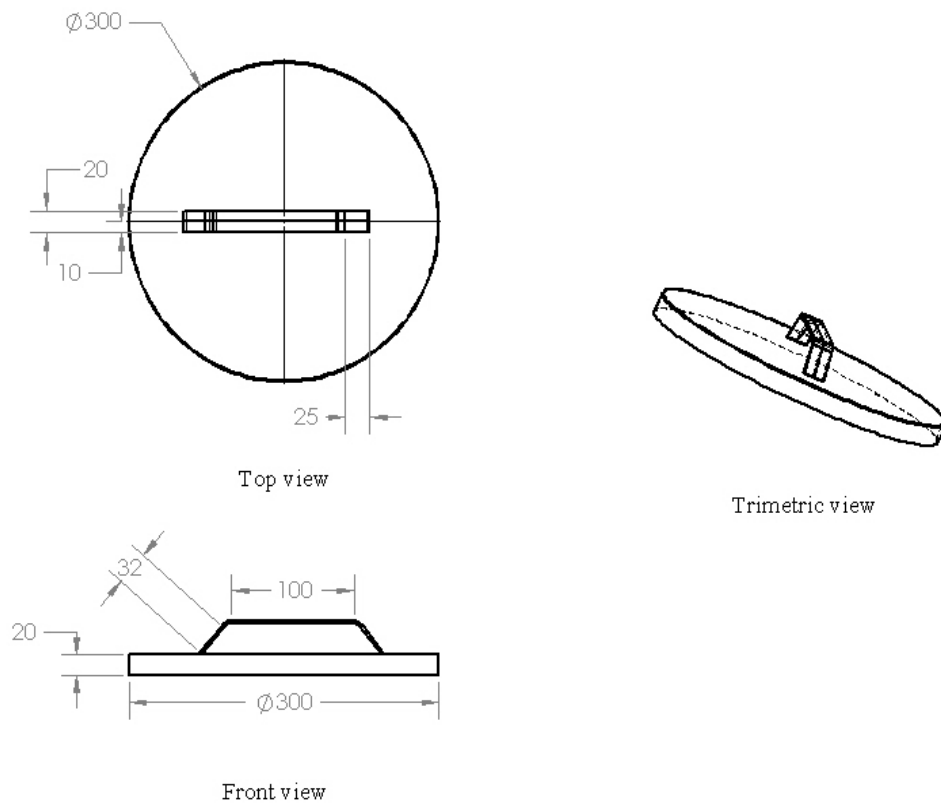
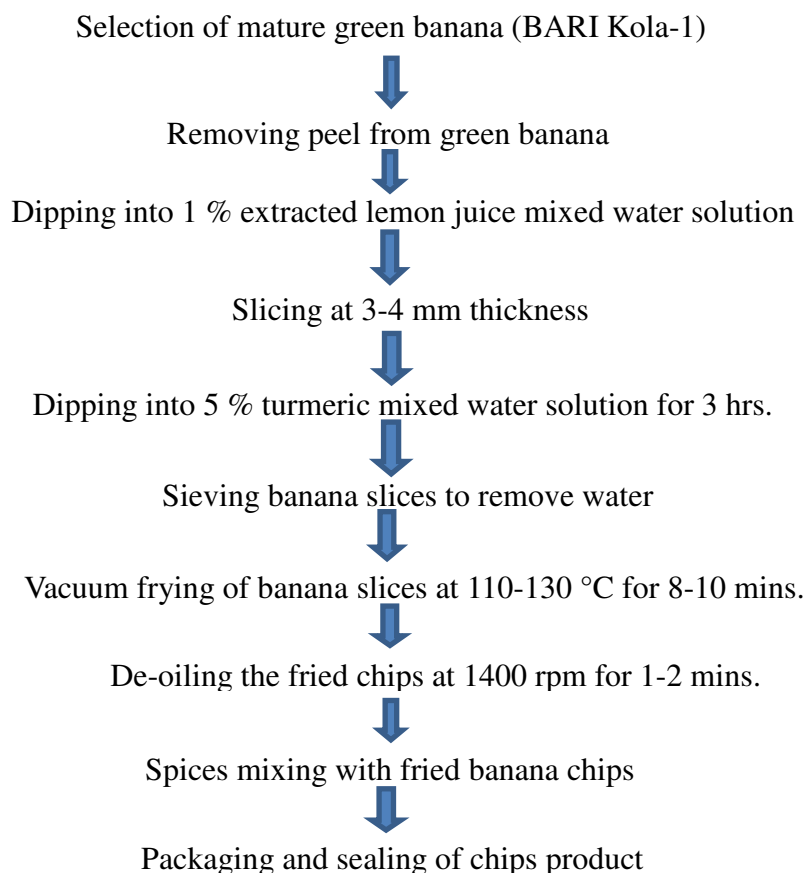


Fig 4. Top, front and trimetric views of the lid for BARI de-oiling machine.

10.5.3. Technology development of the vacuum fried banana chips

10.5.3.1. Protocol of Banana chips processing

Local variety of banana was collected from Harinal bazar of Gazipur Sadar upazilla under Gazipur district and BARI Kola-1 was collected from local farmer of Dhamti village in Debidwar upazilla of Cumilla district. Green unripe bananas were thinly sliced, soaked into lemon and salt mixed water solution and then soaked for 3 hrs in turmeric mixed solution for color development as well as use as natural preservatives (Plate 1). Then the following steps were maintained to prepare vacuum fried banana chips product (Flow diagm 1).



Flow diagm 1. Processing protocol of BARI Vacuum fried Banana chips.



Plate 1. Flow chart for processing of banana (BARI Kola-1) chips.



Plate 2. Different spices combinations of fried chips.



Plate 3. Processing of different spices into mixed spices powder.

10.5.3.2. Suitable spices selection for the fried chips product

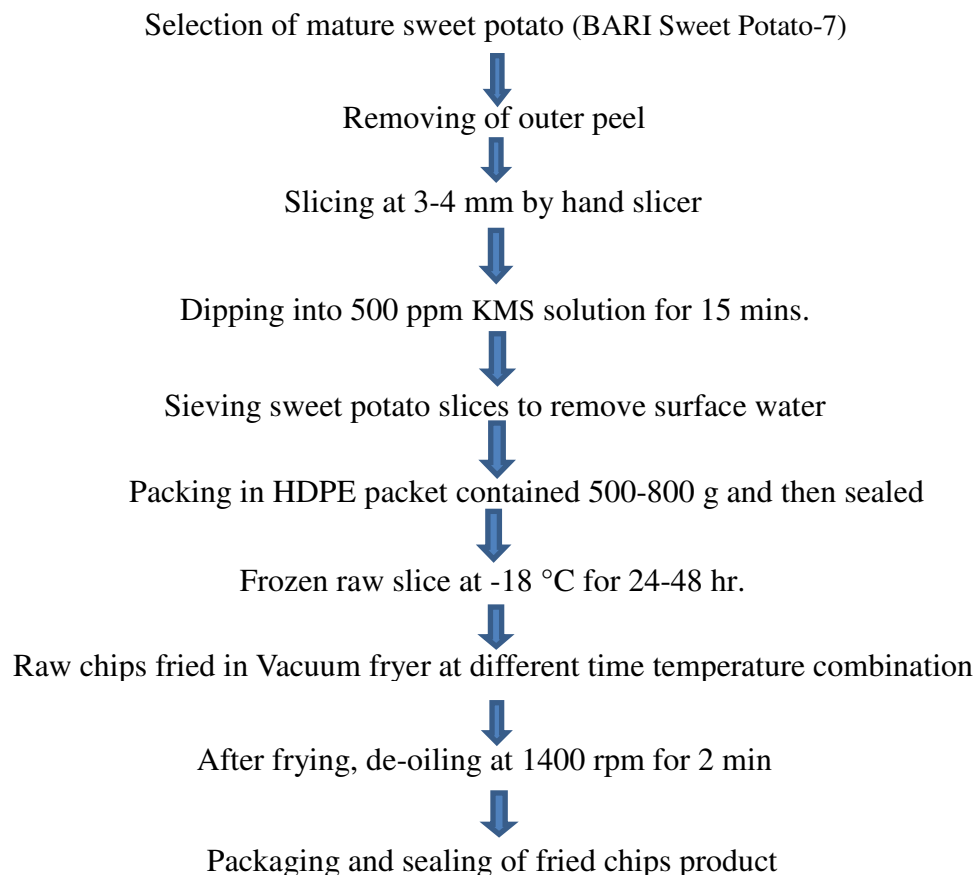
A spice is a seed, fruit, root, bark, or other plant substances which is primarily used for flavoring, coloring or preserving food. For preparing different spices combination for fried chips product, at first salt, sugar, garlic, chili and capsicum fine powder (Sieve size: 80 micron) were processed. Garlic bulbs were separated and peeled. Then the bulb was blanched for 3 mins subsequently and dried at 60-70 °C in the cabinet dryer up to overnight. Red chili and capsicum were not blanched but dried for overnight at the same temperature in the cabinet dryer. After drying, the spices were powdered with proper blending and sieving appropriately. Five various combinations of mixed spices were prepared for vacuum fried chips products as shown in Plate 2 & 3.

10.5.3.3. Sensory evaluation of spices ingredients

For selection of acceptable spices mixture vacuum fried chips product, a consumer panel test was performed which is mentioned in Plate 4. The panelists were comprised of 10 expert persons with different age groups. After scoring with a 0-9 hedonic scale the highest response from the consumers was observed in Type C combination as stated in Table 23.

10.5.4. Technology development of the vacuum fried sweet potato chips

10.5.4.1. Protocol for processing of sweet potato chips



Flow diagm 2. Processing protocol of BARI Vacuum fried Sweet potato chips.

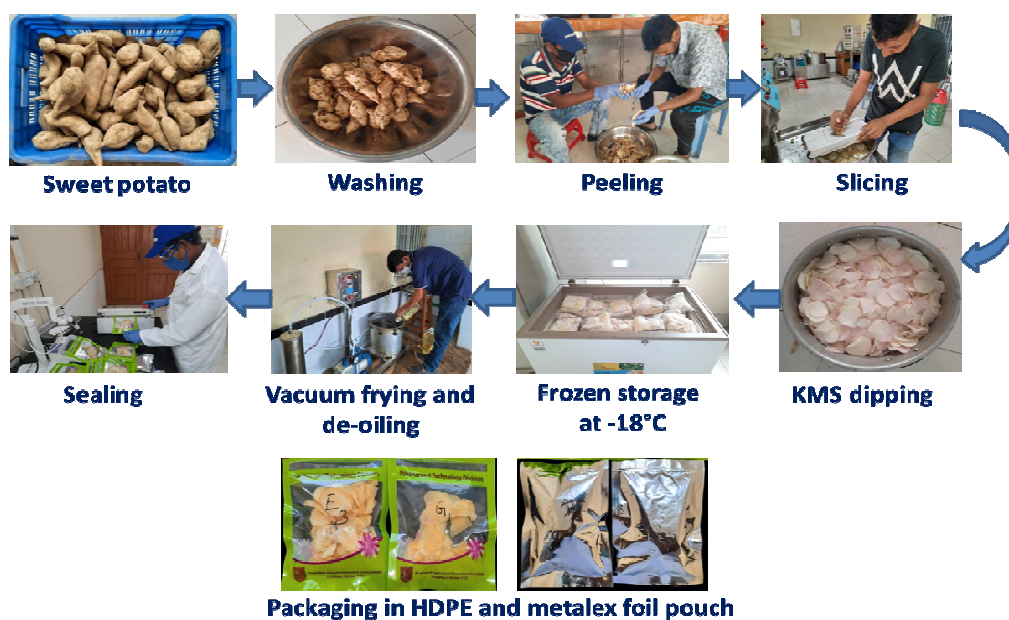


Plate 4. Flow chart of processing for sweet potato (BARI Sweet Potato-7) chips.

10.5.4.2. Experimental trial treatments for developing Vacuum fried Sweet potato chips

At first some preliminary trial was conducted to identify the suitable treatment for sweet potato chips development. Four different treatments were conducted and evaluated by sensory evaluation with expert test panelists. The four treatments are as follows:

- **Treatment- A:** Without blanching + with sugar coating
- **Treatment- B:** Without blanching + without sugar coating
- **Treatment- C:** With blanching + without sugar coating
- **Treatment- D:** With blanching + with sugar coating



Plate 5. Treatment-A & Treatment-B

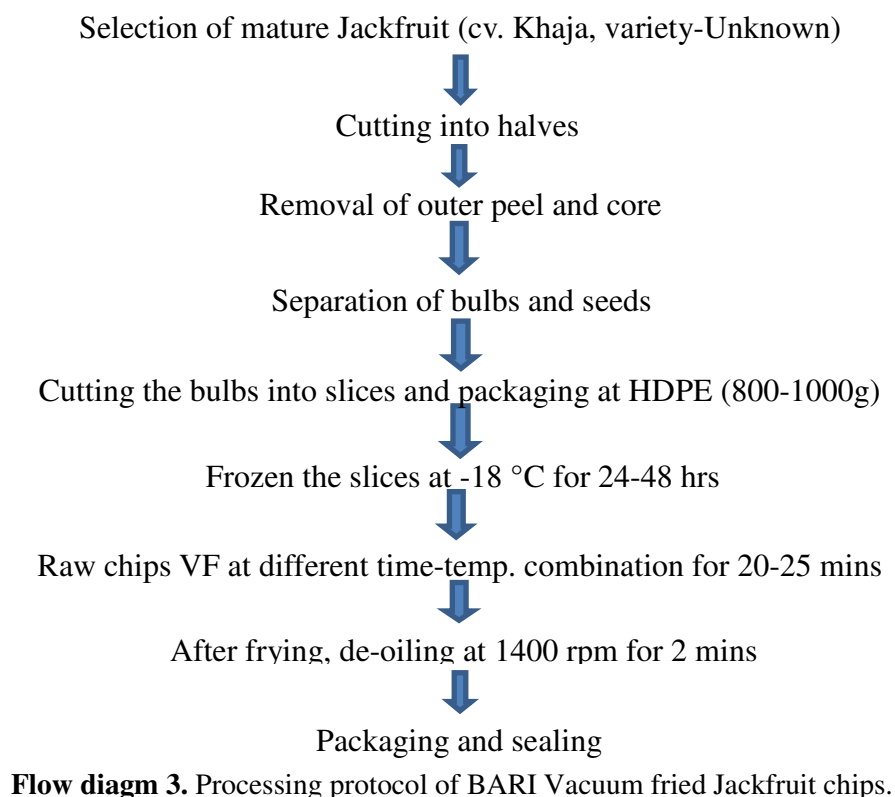


Plate 6. Treatment-C & Treatment-D

10.5.5. Technology development of the vacuum fried Jackfruit chips

10.5.5.1. Protocol of Jackfruit chips processing

Jackfruit is a national and largest fruit among all other fruits in Bangladesh. To add value to the jackfruit, vacuum fried (VF) jackfruit chips processing protocol was developed. Mature Khaja type jackfruit was selected for optimization of suitable frying temperature and time combination with nutritional quality study. Matured jackfruit was collected from the Cotton Research, Training and Seed Multiplication Farm, Sreepur to PHTD, BARI, Gazipur. The following procedure was maintained to develop the vacuum fried jackfruit chips product.



10.5.6. Physico-chemical quality evaluation of the vacuum fried chips product

10.5.6.1. Measurements of external appearance for the vacuum fried chips product

The external color of the vacuum fried chips was evaluated with a Chroma Meter (Model CR-400, Minolta Corp., Japan). CIE $L^*a^*b^*$ coordinates were recorded using D65 illuminants and a 10° standard observer as a reference system. L^* is lightness, a^* (-greenness to +redness) and b^* (-blueness to +yellowness) are the chromaticity coordinates. The a^* and b^* values were converted to chroma [$C = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h = \tan^{-1}(b^*/a^*)$]. Before measurement, the equipment was calibrated against a standard white tile. Three readings were taken at different locations on a group of chips product, using 5 groups from each treatment.

10.5.6.2. Evaluating the moisture content of the vacuum fried chips product

Moisture content was determined according to the method described by Ranganna (2007) with slight modification. Five gram of sample was taken in crucible and was placed in an oven dryer at 75°C for 72 hrs until constant weight attained. Percent moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight}}{\text{Initial weight of sample}} \times 100$$

10.5.6.3. Evaluating the texture/crispiness attribute of the vacuum fried chips product

The textural properties of the vacuum fried chips product were determined to estimate resistance by using a Digital Firmness Tester (DFT 14, Agro Technologies, France) whereby each of 3 slices as a replicate was placed on a sample platform at a constant probe speed of 2 mm s⁻¹ on two sides of the slices. Texture was measured based on millimeters of slices deformation using 8-mm diameter flathead probe. The mean value of each chips product was calculated and expressed in N-m.

10.5.6.4. Determination of ash content in the vacuum fried chips product

The ash content was determined according to the method described in National Food Safety Standard Program (GB 5009: 4-2010) 'Peoples Republic of China' with slight modification Rangana (2007). Three grams chips product sample was taken in a pre-dried weighed crucible and was placed in an oven dryer at 75 °C for 48 hrs. Then the sample was removed and placed in a muffle furnace and heated at 600 °C for 4 to 5 hrs until the sample color turned to completely whiteness. Now the crucible was taken out of the furnace and cooled it in a desiccator carefully and then weighed. Percent ash content was calculated using following formula:

$$\text{Ash content (\%)} = \frac{m_1 - m_2}{m_3 - m_2} \times 100$$

Where, m_1 = Weight of crucible with ash, g
 m_2 = Weight of empty crucible, g
 m_3 = Weight of crucible with fresh sample, g

10.5.6.5. Estimating the water activity of the vacuum fried chips product

The water activity of the vacuum fried chips product sample was determined using the measurement instrument Lab Touch-aw (Novasina Ag) Measurement range, aw: 0.06 ... 0.97 aw (non-condensing) Measurement range temp.: 5 ... 45°C, Sensor: with resistive-electrolytic measurement cell CM-2. Communication: RS-232, USB2x RS-485 for add. Chambers SD card>PC (Nova Log MC). At first sample was blended to make powder form. Then 5g sample was placed into the sample cup and then placed into the sample holder and run the machine. The result was expressed in percentage.

10.5.6.6. Measurement of fat content (oil uptake) in the vacuum fried chips product

The fat content was determined by the Soxhlet Extraction device and the method was followed as described by Ranganna (2007). The fat content was determined quantitatively by gravimetric method by extraction with a mixture of chloroform and methanol (2:1). Five grams of dried powder sample was taken in round joint flask and mixed with chloroform and methanol (2:1). Then, it was incubated at room temperature overnight. Then, filtration was carried out until the color becomes clear (Color of the substance on the filter paper would be colorless). Filtrate was taken in a conical flask of known weight with boiling chips. Then the sample was heated in a chamber until the solvent was evaporated and dried in an oven at 105°C for 3-4 hrs. Finally, weight of the conical flask was recorded.

10.5.6.7. Determination of ascorbic acid content for the fried chips products

The ascorbic acid content was determined according to Ranganna (2007) using 10g samples blended for 2 mins and homogenized with 50 mL of 3% cold meta-phosphoric (HPO_3) acid. Now, the samples were filtered through Whatman filter paper No. 2. The clear supernatant samples were collected for assaying ascorbic acid and then 10 ml of aliquot samples was titrated with 0.1% 2,6-dichlorophenolindophenol solution until the filtrate changed to pink color persisted at least 15 seconds. The titer value was recorded for each aliquot sample. Prior to titration 2,6-dichlorophenolindophenol solution was calibrated by ascorbic acid standard solution. The results were expressed as mg/100g.

10.5.6.8. Determination of total carotenoids content for the vacuum fried chips products

The estimation of carotene was done by the extraction of 3 g sample with acetone and petroleum ether. It was further purified with acetone, metabolic KOH and distilled water. The resulting solution was filtered with anhydrous sodium sulphate and read on a spectrophotometer at 451 nm against petroleum ether as blank. A standard graph was plotted using synthetic crystalline carotene (Fluka, Germany) dissolved in petroleum ether and its optical density measured at 451nm.

10.5.6.9. Estimating the energy content for the vacuum fried chips products

Energy content of fried chips product was determined using bomb calorimeter. One gram sample was taken in a sample cup. Then the sample was put on the head attached with a cotton thread to

the fuse wire and was loaded the head into the bomb cylinder. The oxygen connection was placed and was filled to the bucket with 2-L (2000 ± 0.5 g) water and was placed the bucket inside the calorimeter. The energy content was expressed as calorie/g.

10.5.6.10. Evaluating the sensory attributes of the vacuum fried chips products

For evaluating the changes of the sensory quality attributes of the vacuum fried chips products of different frying temperature and time combination, few panel tests were performed at one month interval during storage period. The sensory panelists comprised of 8 to 10 expert members. After scoring with a 0-9 hedonic scale the highest response was marked and comments of the expert persons were documented for quality improvement.

10.5.6.11. Statistical analysis of the recorded data

All data was expressed in duplicate as means $\pm$ standard deviation. One-way ANOVA with posthoc using Turkey Multiple Comparison Test was performed to analyze the recorded data. The connotation was defined at the 95% confidence level. Statistical analysis and data processing were performed using software SPSS 17.0 (IBM INC., New York).

10.6. Screening and cultivation of some available forage herbs in Bangladesh

For the determination of polyphenolic compounds, different types of herbs were investigated at different area in Bangladesh. Finally, from the literature review, plantain (*Plantago lanceolata* L.), ivy gourd (*Coccinia grandis* L.), Lemon grass (*Cymbopogon citratus*), garlic (*Allium sativum*), spear mint (*Mentha spp*), were detected for the determination of polyphenolic compounds. These herbs were collected from different region in Bangladesh except plantain seed. Plantain seed were imported from New Zealand. All of these herbs were sowed in Shahjalal Animal Nutrition Field laboratory under the department of Animal Nutrition, Bangladesh Agricultural University.

a. Weeding and watering of herbs

The selected herbs named Ivy gourd (*Coccinia grandis* L.), Garlic (*Allium sativum*), Lemon grass (*Cymbopogon citratus*), Mint (*Mentha spp*), and Plantain (*Plantago lanceolata* L.) were planted, weeded by labors and watering by the student. The students supervised and monitored of the herbs every-day. Three times weeding were done due to higher growth of the non-desert weeds at the herbs plot. Watering was done two times from the winter season and up-to rainy season. Proper care was also taken as the water is not logged at the herbs plot. The drainage system was cleaned sometimes.



Plate 7. Weeding and watering of selected potential herbs.

b. Screening of phytochemicals of selected medicinal herbs

Thin layer chromatography (TLC) is an important technique for identification and separation of mixtures of organic compounds. In TLC, components of the mixture are partitioned between an adsorbent (the stationary phase, usually silica gel, SiO_2) and a solvent (the mobile phase) which flows through the adsorbent. For comparison of extracted residues with routinely used standard phenolics were prepared (Sigma Co.) were prepared by dissolving of 0.1 g of each Powder in 4 ml methanol. Preliminary qualitative phytochemical screening was carried out with the following methods (Shanmugam *et al.*, 2010)

c. Pointing, running and detection

A volume of 50 μl of methanol dissolved deposits was pointed on silica plates. Treated plates transferred to TLC tank containing acetone-methanol (1:1) which is mobile for TLC. After reaching of solution front to end of plates, chromatograms observed on ultraviolet light at 256 nm.



Plate 8. Phytochemical screening of medicinal herbs.

10.7. Selection, harvesting and preparation of selected herbs for broiler and layer feeding trail

Based on TLC result, biomass yield, mineral contents and specific performance on livestock, plantain, ivy-gourd, spear mint, lemongrass, and garlic leaves were harvested. Plantain herbs had growth promoting effects on livestock due to contain acteoside, aucubin and catalpol bioactive components. Ivy-gourd are rich source of minerals and bioactive components. Garlic leaves contain sulphur compounds and allin which are responsible for immunity and growth of livestock. Due to contain aromatic compounds in mint is responsible for good aroma in broiler's meat.



Plantain herb harvesting

Plate 9. Plantain herb harvesting.

a. Preparation of herb mixture powder

With a view to making the sustainable and year round herb supplementation technology in broiler diet, the herbs are supplied into two forms i) Shade dried forms. ii) Blanched forced dried forms.

b. Preparation of shade dried powder

At first plantain herbs, garlic and spearmint were cultivated and dried under shade by continuous air supply at 25-30⁰ C in separated polythene. When the herbs were completely dried and fit for grinding and then grinded by grinder machine.

c. Preparation of blanched powder

Blanching is technique to encounter the mechanism of enzymes and keep the herbal bioactive components mostly intact. In this process, the ice was collected from Machuya Bazar, Mymensingh city. This ice was submerged and broken in a large container containing water and taken the temperature by special thermometer. Besides, the water in large container were boiled and taken temperature with the help of special thermometer. On the other hand, the herbs were cut and submerged into hot water at 95-100⁰C for 5s seconds and immediately cold water containing ice at 0-5 ⁰C for 0-5 seconds. The ice was added to keep the cold water temperature constant and another container was heated to keep the temperature constant. The wet herbs were dried in hot air drier at 50-60 ⁰C with close inspection and regular interval of handling. Finally, the herbs were grinded with the help of grinder machine to prepare fine powder.

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Plate 10. Blanching and shade drying of herbs.

d. Broiler feeding trial

Determination of threshold level of herb mixture for broilers

Broiler feeding trial was conducted for assessing the impact and determination of appropriate concentration of herb mixture in broilers. The herb mixtures are composed of plantain herb, ivy-gourd, mint, and garlic leaves at a fixed ratio. The research study was designed as four experimental treatments using formulated diet with 0.0% (T_0), 0.5% (T_1), 1.0% (T_2), and 1.5% (T_3) herb mixture supplementation on DM basis. Among four treatments, T_2 (1.0% herb mixture) group showed highest live weight gain, better FCR, plasma metabolites and carcass characteristics of broilers compared to T_3 , T_1 , and T_0 group, respectively. It might be concluded that 1.0% herb mixture on DM.

e. Ration formulation for broiler diets

The broiler diets were formulated at Minar Feed Mill, Trishal as crumble and pellet forms for grower periods and starter period, respectively. The herbs mixture was added in micro-ingredients and well mixed for homogenous distribution. The micro-ingredients were L-lysine, DL-methionine, toxin binder, SQ-enzyme, DCP, Sodium bi-carbonate, phytase enzymes etc. The macro-ingredients named maize crushed, protein concentrate, soybean meal, rice polish, and oil were well mixed for making homogenous mixture. By using both ingredients the pellet feed was prepared for starter and grower period and then pellet was sieved for grower period as crumble form. Feed (pellet form) was fed to the experimental birds. The chemical composition of commercial feed and formulated feed were alike (Table 1). In lieu of herb mixture the antibiotic growth promoters (AGPs) were added in broiler diets. The formulated feed were sent to Manikganj via truck and brought to Animal Nutrition lab for proximate analysis. (Table 2).

Table 1. Amount of different ingredients used in formulated diets for T₀ and T₁ and T₂ groups

Ingredients name	Amount (%)		
	Formulated diet + AGPs (T ₀)	Formulated diet + shade dried herb mixture (T ₁)	Formulated diet + blanched forced dried herb mixture (T ₂)
Maize	53	53	53
Protein concentrate	7	7	7
Soybean meal	30.6	30.6	30.6
Rice polish	3.2	3.2	3.2
Oil	3.2	3.2	3.2
DCP	0.4	0.3	0.3
Limestone	1	1	1
Lysine	0.3	0.3	0.3
Methionine	0.2	0.2	0.2
NaHCO ₃	0.1	0.1	0.1
Choline chloride	0.1	0.1	0.1
Enzyme TS	0.02	0.02	0.02
Toxin binder	0.1	0.1	0.1
Anti -salmonella killer	0.1	0.1	0.1
Broiler Premix	0.21	0.21	0.21
Salt	0.22	0.22	0.22
Phytase enzyme	0.05	0.05	0.05
CTC growth promoter	0.10	0.00	0.00
Growth promoter	0.05	0.00	0.00
Toxol	0.05	0.05	0.05
Herb mixture (90PL +5IG + 3 GL +2 SM)	0.0	1.0	1.0
Total	100	100	100

Table 2. Chemical composition of formulated diet (g/100g DM)

Proximate components	Amounts %		
	Formulated diet + AGPs (T ₀)	Formulated diet + shade dried herb mixture (T ₁)	Formulated diet + blanched forced dried herb mixture (T ₂)
Dry matter	89.00	89.00	89.00
Crude protein	22.25	22.30	22.30
Crude fibre	4.86	4.99	4.99
Ether extract	3.98	3.98	3.98
Nitrogen free extract	49.52	48.74	48.74
Ash	8.39	8.99	8.99
Calcium	0.93	0.98	0.98
Phosphorus	0.49	0.51	0.51
ME (Kcal/kg)	2985	2985	2985



Plate 11. Ration formulation.

f. Animal Ethics and location of the experiment

The whole experimental procedures, animal handling, collection of shank and meat sample were reviewed and approved by the animal care committee of Bangladesh Agricultural University Research System (BAURES). The research work was carried out with 3024 straight run day-old broiler chicks (Cobb-500) for a period of 32 days to investigate the effects of medicinal herb mixture composed of plantain herb (*Plantago lanceolata* L.), ivy-gourd (*Coccinia grandis*), spearmint (*Mentha spp*), garlic leaves (*Allium sativum*) on growth performance, feed conversion ratio, shank mineralization, meat fatty acid specially ω -3 fatty acid level, and meat colour of broiler's meat.

g. Experimental design and layout

The experiment was conducted in a Completely Randomized Design (CRD) containing 3024 Chicks were randomly distributed into 3 equal groups with 3 replicates in open sided shed house each replication having 336 chicks (i.e. $3 \times 3 \times 336 = 3024$ birds) Three (3) dietary treatment groups are shown in Table 3.

Table 3. Layout of the experiment

Replication	Treatments			Total No. of birds
	T ₀	T ₁	T ₂	
R ₁	336	336	336	1008
R ₂	336	336	336	1008
R ₃	336	336	336	1008
Total	1008	1008	1008	3024

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture
Herb mixture was composed of Plantain herbs, ivy gourd, mint, and garlic leaves.

10.8. Broiler bird's husbandry

a. Preparation of shed

The experimental room (ceiling, wall, floor, and wire net) were properly brushed with the help of a broom and washed and cleaned by forced water, then floor of the house was disinfected by using phenyl solution. Room was left vacant for 7 days. Ceiling wall and wire nets were thoroughly disinfected by spraying Vircons-S solution@ 1g/L water (Antec International Limited, England) and kept free to dry up properly. At the same time, all feeders, plastic buckets, drinkers, and other necessary equipment were also properly cleaned, washed, and disinfected with bleaching powder solution, subsequently dried and left them empty for one week before the arrival of the chicks.

b. Procurement of chicks

A total of three thousand twenty-four (3024) day-old broiler chicks (Cobb-500) were distributed in three different dietary treatments. After arrival of chicks in each experimental house, they were supplied 5% glucose solution to minimize stress for transportation. The chicks were distributed randomly to individual group one day after arrival according to treatment.

c. Light, temperature and ventilation

For light and temperature control, twenty 100-watt electric bulbs were used for each shade. Lighting periods of 24 hours per day were provided throughout the experimental period. At the early stage, bulbs were hanged just above the chick's level. The brooding temperature was maintained at 34°C for the first week. Temperature was measured with the help of Clinical Thermometer. Humidity of the entire room was maintained 65 to 68. Wire net was used for maintaining the ventilation. In the course of trial, temperature was gradually reduced at the end the trial by using electric fans.

d. Feed and water supply

Feed in the form of crumble was supplied to the chicks on clean newspaper for the first 12 days and water was supplied in round plastic drinkers from the first day. After 3 days of age, feed was supplied in round feeder with a capacity of 2 kg feed and round drinker with a capacity of 4 litres

water. Feeds and water were offered on ad libitum basis to the experimental chicks according to separate dietary treatments.

e. Litter management

Bamboo slat was used instead of litter material in each experimental farm. The gaps between the planks of the bamboo slat facilitated to drop down the poultry droppings on ground under the slat. Poultry droppings was cleaned at every two alternate days.

f. Vaccination

The broiler is susceptible to various diseases like New Castle disease and Infectious Bursal Disease. All the experimental chicks were vaccinated against Infectious Bursal Disease and New castle disease. All the vaccines were administered as per recommendation of the manufacturer. Adequate precautions were also taken in case of vaccination. The vaccination schedule followed during the experimental period is given below in Table 4.

Table 4. Vaccination schedule for the experimental broilers

Age	Name and type of the vaccine	Name of disease	Route of administration
5th day	ND	Newcastle disease	Eye drop
12th day	IBD	Gumboro disease	Eye drop

g. Bio-security

Proper bio-security measures were taken during the experimental period for reducing the mortality rate and to produce healthy birds. Equipment was made clean and disinfected. Footbath (containing solution of potassium permanganate) was at the entrance of the experimental house. Before entrance, shoes were changed, and feet were dipped in a footbath. Apron, mask, hand gloves were used inside the house during handling the birds, giving feed and water to the birds, and collecting sample from the birds. Dead birds were buried from the experimental house and sick birds were promptly isolated to a separate place from the experimental house. Strict bio security program was maintained during the whole experimental period.

h. Regular check-up and clinical observation

The chicks were observed twice a day for any physical abnormalities (slow movement, infrequent sitting, lack of appetite, paralysis etc.). Other activities were also monitored.

i. Record keeping and data collection

The following records were kept during 32 days of the experimental period:

j. Growth performance

Growth performance was recorded from each of the replication after ending of every eight (8) days.

k. Live weight

Chicks were weighed in a group for each replication during onset of the trial and at the age of 0, 8, 16, 24 and 32 days using a digital electronic top pan balance.

l. Calculation of body weight gain

The average body weight gain of chicks in each replication was calculated by deducting initial body weight from the final body weight.

Weight gain = Final live weight - Initial weight

m. Feed consumption

Feed consumption was recorded by subtracting refusal from the supplied feed divided by the number of birds at the end of each week per replication.

n. Feed conversion ratio

Feed conversion (feed: gain ratio) was calculated as the ratio between feed intake and BW for each period.

o. Mortality

The mortality of the chick in each replication from a treatment was recorded when death occurred.

10.8.1. Slaughtering and dressing of broiler

At the end of the experimental period (32 days), five broilers from each replication was slaughtered for determining dressing yield. Broilers were selected based on mean weight of each replication. Feed was withheld overnight before slaughter. Broilers were identified using number on leg band. Broilers were slaughtered by Halal method and allowed them to bleed for 2 minutes. The slaughtered birds were weighed after totally expelled of blood and immersed in hot water (51-55°C) for 120 seconds in order to removal of feathers and cut into carcass parts in accordance with the procedures as outlined by (Kim *et al.*, 2011). Head and shank were separated; viscera and gizzard (heart, liver, spleen, and gizzard) were removed from the carcass.

10.8.2. Collection of meat and shank sample

Approximately, 100 g of meat sample for proximate analysis were taken from breast region of each bird. The meat sample were weighted carefully and packed with marking separately. The

shank and meat sample were collected from the slaughtering spot and transported to BAU, by maintaining cold chain and stored as -20°C .

10.8.3. Chemical analysis

Sample feed ingredients were analysed to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE) and total ash (ASH) following the method described by AOAC (2005).

10.8.3.1. Broiler meat fatty acid determination

Fatty acid composition was analyzed by Gas Chromatograph fitted with Flame Ionization Detector (GC-FID), Model 14B SHIMADZU, Japan, loaded with software Class GC-10 (version-2.00). The GC was equipped with Flame ionization detector (FID) and capillary column (Restek® CATcc483, Serial No. 1324355, USA), with dimension 15m length and 2.25 mm ID. The operating condition was programmed at oven temperature 150°C (holding time 5 min), $8^{\circ}\text{C}/\text{min} - 190^{\circ}\text{C}$ (holding time 0 min), $2^{\circ}\text{C}/\text{min} - 200^{\circ}\text{C}$ (holding time 10 min), injection port temperature and detector temperature were 250°C . Nitrogen was used as carrier gas with the flow rate of 20 mL/min. 10 mL of clarified fat was taken in 15 mL test tube and 3 mL of 0.5 M sodium methoxide (prepared by mixing metallic sodium in methanol) was added and digested by stirring in a boiling water bath for about 15 minutes. It was allowed to cool to room temperature and 1 mL of petroleum ether (boiling point, $40-60^{\circ}\text{C}$) was added followed by adding 10 mL of deionized water, mixed gently, and allowed to settle for 2-3 min. The distinct upper layer of methyl ester in petroleum ether was separated carefully in a capped vial and used for analysis. Two hundred mg of $\text{C}_4\text{-C}_{24}$ fatty acid (both saturated and unsaturated fatty acid) standard (Restek®, USA) in their respective methyl ester form were dissolved separately in 10 mL petroleum ether (boiling point, $40-60^{\circ}\text{C}$) in a series of screw capped test tubes. Aliquots of 1 μL FAME were injected, and the peaks of fatty acids were recorded for their respective retention time and areas by the data processor unit of gas chromatography.

10.8.3.2. Meat colour determination

The colour of meat including breast and thigh meat were determined after immediately slaughtering the broiler with Minolta CR-400 colourimeter (MINOLTA CAMERA Co. Ltd., Osaka, Japan) calibrated against white plate ($L^*=93.30$; $a^*=0.32$ and 1.8 ; $b^*=0.33$) with 8 mm optical probe diameter, D65 illuminant and 2° observer. The meat colour is presented as CIE- $L^*a^*b^*$ (Commission Internationale de l'Eclairage, 1976).

10.8.3.3. Shank mineral determination

To determine bone ash content, bones were oven-dried at 105°C for 24 h. The samples were weighed after drying (DM), placed in a pre-weighed crucible and ash in a muffle furnace at 600°C for 6 h and then re-weighed (ASH, g/100 g DM). The total phosphorus content of the bone and feed was determined by using an auto analyser and the calcium was measured by atomic absorption spectrometry at central lab, BAU.

Statistical analysis : Data were represented as the mean $\pm$ SD (standard deviation). At first the raw data were organized using computer Excel program and then analyzed using SPSS (2002) statistical program for one-way analysis of variance (ANOVA). The significance of difference among means was determined using Tukey's HSD test (1953) and differences at $P < 0.05$, $P < 0.01$ were considered statistically significant.

10.9. Layer feeding trial

10.9.1. Herbs cultivation and preparation for feeding trial

The selected herbs viz. plantain (*Plantago lanceolata* L.), spear mint (*Mentha spicata*), ivy-gourd (*Coccinia grandis*), garlic leaf (*Allium sativum*), and lemongrass (*Cymbopogon citratus*) cultivation are being continued with performing proper weeding and watering at Herbal Germplasm centre, Shahjalal Animal Nutrition Field Laboratory, under the Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh. The plantain herb, mint, garlic, and ivy-gourd leaf were harvested just before blooming stage, at mature stage, during harvesting of garlic cloves, and before flowering stage for layer trial, respectively. In previous trial, different forms of herb including shade dried, sun dried, and blanched dried powder were offered to the broiler at 1.0% threshold level, where 1.0% shade dried herb mixture was showed better result compared to rest forms of herb mixture powder. So, plantain, mint, ivy-gourd, and garlic leaf powder were dried at shade at 30-35°C to minimize the fungal, mould or bacterial proper wilting was performed after 6 hours interval. The shade dried herb mixture was grinded by grinder machine at Shahjalal Animal Nutrition Field Laboratory for Layer trial and kept at normal temperature. Besides, the plantain and ivy-gourd herb were selected for sheep trail. The threshold level of herbs in sheep were determined by the fund of CRG, MOP and including this. The sheep was showed better performance at 10g DM of plantain herb, 10g DM of garlic leaf and 5+5 g DM of plantain and garlic leaf, this research was published at Journal of Animal in last issues. From these, 10g DM of plantain and 10g DM of ivy-gourd, both herbs at 5g DM of plantain and garlic leaf were offered to the sheep, as no previous study was not performed on sheep about mutton quality and osteoporosis.

10.9.2. Experimental design, diets, housing, and managements

The experimental procedures, bird handling, collection of egg and blood samples have been reviewed and approved by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University. A total of 360-day old Lohmann Brown chicks were procured at Minar Feed Mill, Kanhore Bazar, Trishal, Mymensingh. At 32 weeks old of Lohmann Brown laying hens with uniform body weight were randomly allocated at 60 cages in a completely randomized design until 40 weeks of age (experimental period 8 weeks). The laying hens were assigned into 5 treatments replicated 5 times, containing 12 birds/replication or cage and offered a corn-soybean-basal diet in mash form as control group without herbal supplementation. The rest treatments were CON+5 g HMP/Kg diet, CON+10 g HMP/Kg diet, CON+15 g HMP/Kg diet, and CON+20 g HMP/Kg diet. The herb mixture powder (HM) was contained dried plantain, ivy-gourd, mint, and garlic powder. The ingredients, the nutrients concentration and chemical composition of control diet were shown in Table 5 (AOAC 1995). The experimental diets were formulated to meet the nutrient requirements for layer hens

according to the recommendations of the breeder (Lohmann Brown Lite layers, Commercial Management Guide, Lohmann Tierzucht, Cuxhaven, Germany). Feed and water were provided ad libitum during the experiment. The experiment was carried out between 32 and 40 weeks of age (8 weeks experimental period). Hens were housed in open sided equipped with feeders and nipple drinkers. The hens were maintained under a 16 hour (06:00 to 22:00) light and 8 hour dark schedule per day. The laying hens were kept in optimal and standard temperature.

Table 5. Ingredients and chemical composition of control diets

Items	Amounts
Ingredients	%
Maize	55.75
Soybean meal	19
Protein concentrate	6.6
Rice polish	6.5
Limestone	9
Di-calcium phosphate ¹ (DCP)	1
Oil	1.2
Layer premix ²	0.2
Lysine	0.1
Methionine	0.2
Coccidiostat	0.1
Toxin binder	0.2
Salmonella Killer	0.01
Rena-phytate enzymes	0.02
Salt	0.25
Calculated chemical composition	g/100g
DM	88.27
CP	17.96
CF	4.24
EE	3.59
Ash	10.14
NFE	64.07
Ca	3.70
P	0.38
Lysine	0.92
Methionine	0.52
Linoleic Acid	1.39
ME (Kcal/Kg)	2770.90
Analysed chemical composition	g/100g
DM	88.04
CP	17.47
CF	4.03
EE	3.57
Ash	9.78
NFE	65.15
Ca	3.62
P	0.35

¹8% granular phosphate and 23% calcium.

²Vitamin A 12,000 IU, vitamin D3 3,000 IU, vitamin E 40 mg, vitamin K3 3 mg, vitamin B1 2 mg, vitamin B2 6 mg, vitamin B6 5 mg, vitamin B12 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg. ³DL-methionine, Met AMINO (DL-2-amino-4-(methyl-thio)- butane acid, DL-methionine, α-amino-γ-methyl-oily acid) by Feed Grade 99% (EU).

a. Record and sample collection

A total of 30 eggs/treatment (6 eggs/ replication) were taken weight daily basis for measuring the egg weight, egg mass, feed conversion ratio (FCR). The feed intake was measured daily, and hen day egg production was used to determine the performance of the flock. At the end of 36 week of age, a total 15 eggs (3 eggs/replication) were collected and brought to Animal Nutrition laboratory for further analysis within 24 hours maintaining room temperature. At the end of 40 weeks of age, in total 30 eggs/treatment were collected and subdivided two groups for analysing the external and internal egg quality (15 eggs) and for measuring the level of antioxidants (15 eggs). The collected 15 eggs for measuring egg quality was stored at room temperature and all analysis were performed within 24 hours. The rest egg sample were stored at -20°C until analysis. At the last day of 40th week of age, the blood sample (10 birds/treatments) were collected and centrifuged 3421×g RCF for 15 min for plasma separation and then stored at -20°C until analysis.

b. Sample analysis

The feed consumption and feed conversion ratio were recorded in 7-d intervals. The feed conversion ratio (FCR) was expressed as grams of feed consumed per grams of egg produced. Egg mass was calculated by multiplying egg weight by egg production. All production variables were determined on a replicate basis.

10.10. Determination of egg external quality

The egg length (L) and diameter (d) were taken using slide calliper to the nearest 0.01mm. The egg shape index (SI) was measured from egg length and diameter according to Readdy et al. (1979) and Anderson et al. (2004) as given with the following formula:

$$\text{SI} = (d / L) * 100$$

According to this the eggs were classified with respect to shape index (SI), namely as a sharp egg ($\text{SI} < 72$), a normal (standard) egg ($\text{SI} = 72\text{--}76$) or a round egg ($\text{SI} > 76$) (SARICA And ERENSAYIN, 2009).

The egg surface area measured using the following relationship cited by (CARTER, 1975), where S is the surface area in cm^2 and EW is the egg weight in g.

$$S = 3.9782 \times \text{EW}^{0.7056}$$

The eggshell thickness was measured according to CHOWDHURY, 1990 by using a digital micrometer. The weight of eggshell was taken after removing the shell membrane and shell percent was calculated by adopting the following formula, SW and EW are the shell and egg weight in g, respectively.

$$\% \text{ shell} = (\text{SW}/\text{EW}) * 100$$

a. Determination of egg internal quality

Egg albumen and yolk height was determined by using a tripod micro-meter in the Animal Nutrition Laboratory. The diameter of yolk was measured by slide calliper. The diameter of thick albumen was taken from two side of the albumen and finally average value was calculated for determining the albumen index (AI). The AI was calculated using albumen height (Ah) is divided by albumen diameter (Ad) and multiplied by 100. Besides, Yolk index is related to the yolk height (Y_h) and yolk diameter (Y_d). Moreover, Haugh unit (HU) is related to the albumen height and egg weight. Individual Haugh unit (Haugh, 1937) score was calculated using the egg weight and albumen height (Doyon et al., 1986). The Haugh unit (HU) values were calculated for individual eggs using the following formula, where H is the observed height of the albumen in mm and EW is weight of the egg in g (MONIRA et al., 2003).

$$\text{Albumen index} = (\text{Albumen height} / \text{Albumen diameter}) * 100$$

$$\text{Yolk index} = (\text{Yolk height} / \text{Yolk diameter}) * 100$$

$$\text{Haugh unit} = 100 \log_{10} (\text{Albumen height} - 1.7 \times \text{Egg weight}^{0.37} + 7.6)$$

The blood and rest egg samples were kept at -20°C for further analysis due to COVID-19 and fund crisis.

b. Statistical analysis

Data were represented as the mean $\pm$ SD (standard deviation). At first the raw data were organized using computer Excel program and then analyzed using SPSS (2002) statistical program for one-way analysis of variance (ANOVA). Significant differences among the treatments were determined using Tukey's test at $P < 0.05$.

10.11. Sheep feeding trial using plantain herb and garlic leaves

A. Material and methods

a. Experimental animals

The experimental procedures, animal handling and the collection of blood, rumen tissue, and meat sample were reviewed and approved by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University (AWEEC/BAU/2019/31). A total of 32 non-descriptive sheep (9 ± 0.2 kg) indigenous to Bangladesh and approximately at the age of 1 year old were allocated to four groups of eight animals. The experiment adopted a completely randomized design. Experimental sheep were reared in individual disinfected cages during the experimental period. Each cage was 0.91 m^2 (120 cm $\times$ 76 cm). Prior to the 14-day feeding trial, sheep were treated with Almex-Vet® tablets (Square Pharmaceuticals Ltd, Bangladesh) orally at a dose of 7.5 mg/kg body weight for gastrointestinal nematode control. The feeding trial lasted for 90 days, which consisted of 15-day adaptation and 75-day main experimental period.

b. Experimental diet

Rice straw, roadside grass, wheat bran, mustard oil cake, molasses, and salt based total mixed ration (TMR) pellets (ME = 2390 kcal/kg DM, CP = 15.1%) were provided as the control diet (CL-diet). Treatment diets consisted of the (i) CL diet + 10 gDM of plantain herb (PL-diet); (ii) CL diet + 10 gDM of garlic leaf (GL-diet); and (iii) CL diet + 5 gDM of plantain herb and 5 gDM of garlic leaf (PG-diet).

Grasslands Lancelot plantain and BAU-3 local garlic genotype were used in this experiment. Fresh plantain and garlic leaves were harvested from different experimental plots at the pre-blooming stage (~60–70 days) and supplied to sheep in addition to TMR pellets. Chemical composition and nutritive values of plantain herb and garlic leaf are presented in Table 6. In all dietary treatment groups, the TMR pellets provided were maintained at 1.5 times the calculated maintenance level (NRC, 1985). Daily required TMR pellets were equally divided into two parts and supplied at 08.00 h and 16.00 h. Clean fresh water was provided ad libitum.

c. TMR pellet preparation

All ingredients (Table 6) for pellet preparation were collected from a local market, and before grinding, the straw and grass were air dried (~30–35°C) for 1 and 3 days, respectively. Then, mustard oil cake, dried grass, and straw were ground with a locally made grinder fitted with a 1-mm sieve. Then all the required ingredients were thoroughly mixed at 1450 rpm for 15 minutes using a locally made paddle-type mixer machine. Besides, 10% water was added to the ingredients at the time of mixing, and then TMR pellets were prepared using a locally made semi-automatic pelleting machine with a 6-mm die.

d. Records and sample collection

Daily feed intake was measured based on the difference between the offered and residual feed. The body weight of individual animals was measured weekly, and total gain was calculated based on the difference between the initial and final body weights. The feed conversion ratio (FCR) was determined cumulatively through the collected data. Faecal samples were collected during the last 7 days of the experimental period. Following daily collection, faecal samples were weighed, and thoroughly mixed, and representative samples were stored at –8°C. After 7 days of collection, faecal samples were thoroughly mixed and oven-dried at 65°C for 72 hours. The faecal samples were then ground to pass through a 1-mm mesh and stored at –20°C until analysis. Blood samples (5 ml each) were collected on day 89 into a Na-heparinized tube and centrifuged (HERMLE Z 306) at $3421 \times g$ RCF for 15 minutes for plasma separation and then stored at –20°C until analysis. At the end of the trial (day 90), sheep were slaughtered using the halal method to collect meat and rumen tissue samples. Rumen tissue was washed immediately upon collection in phosphate buffered saline and placed in an ice box. Then, tissue samples were dehydrated in 4% formalin solution and ethanol for histological examination. Fore-limb, hind limb, neck, fore-leg, hind-leg, caul fat and pelvic fat were dissected from carcasses following the method of Mahgoub and Lodge (1994). Meat samples for chemical analyses, i.e. proximate composition, fatty acids profile, drip loss, and cooking loss, were taken from the left side of the

carcass (*Longissimus dorsi*) of each slaughtered sheep. Meat samples were stored at -8°C until further analysis.

e. Sample analysis

All the test parameters of feed, faeces, blood and meat samples were analysed in triplicate. Proximate components of plantain, garlic leaf, feed, faeces, and meat samples were analysed using the AOAC (1995) methods. Acid detergent fibre and neutral detergent fibre of plantain, garlic leaf, and feed samples were determined according to Georing and Van Soest (1970). In-vitro organic matter digestibility, digestible energy, metabolizable energy of plantain and garlic leaf were determined according to Menke (1988). Calcium, magnesium, phosphorus, and sulphur content of plantain and garlic leaf were determined using a UV-spectrophotometer (T60, PG Instruments, UK); potassium using a flame emission spectrophotometer (Model-Jenway PFP 7 Industrial flame photometer, UK); and iron, copper, and zinc using a flame atomic absorption spectrophotometer (Model no: SHIMADZU, AA-7000, Japan). The concentration of glucose, urea nitrogen (BUN), albumin, globulin, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), and alkaline phosphatase (ALP) in blood plasma were measured using different enzymatic kits in a bio-analyser (Urit-810, URIT Medical Electronic Group Co, Ltd, China). Serum concentrations of immunoglobulins A (IgA), G1 (IgG1), G2 (IgG2) and M (IgM) were determined using sheep ELISA kits (Sigma-Aldrich, USA). Total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in serum were determined using a UV-Spectrophotometer (T60, PG Instruments, UK) with commercially available kits (Sigma-Aldrich, USA). The length and width of rumen papillae were measured using a light microscope (LABOMED Lx400, with iVu5100 camera module, USA) at $4\times$ magnification. The mutton fatty acid profile was analysed using a gas chromatograph fitted with a flame ionization detector (GC-FID; Model 14B SHIMADZU, Japan) loaded with software Class GC-10 (version-2.00). The GC-FID was fitted with a capillary column (Restek® CATcc483, Serial No. 1324355, USA) 15 m long and 2.25 mm ID. The percentages of mutton drip loss and cooking loss were measured following the method described by Joo (1995) and Aaslyng et al. (2003), respectively. To measure eye muscle area, the hot carcass was split between the 13th and 14th ribs. From the cross section, the area was traced three times onto acetate paper. Then, from the weight–area relationship of the acetate paper, the average area of each single ‘eye muscle’ was estimated using following formula:

f. Eye muscle area (%)

$$= \frac{\text{Weight of acetate paper for total eye muscle area}}{\text{Weight of acetate paper for one cm}^2}$$

Statistical analysis : Results are presented as mean $\pm$ standard deviation. All data were subjected to one-way ANOVA, and the significance of differences among means was determined using the Duncan multiple range test and differences at $P < 0.05$ were considered statistically significant. All analyses were conducted by IBM SPSS 2011 (Version 20.0; New York, IBM Corp, 440) using the following statistical model:

$$Y_{ij} = \mu + t_i + e_{ij}$$

where, Y_{ij} was the response due to subject sheep (i) and treatment (j); μ the overall mean; t_i was the effect of herbal supplementation (treatment effect); e_{ij} was the error due to the j^{th} replication of the i^{th} treatments and normally distributed with zero mean and constant variance.

Table 6. Dietary ingredients and nutrient composition of total mixed ration (TMR) pellet

Ingredients	Amounts (%)
Roadside grass*	27.0
Rice straw	16.0
Wheat bran	30.0
Mustard oil cake	15.0
Molasses	11.5
Common salt	0.50
Calculated chemical composition of pellet (g/100g DM)	
Moisture	9.62 $\pm$ 0.1
Crude protein	15.07 $\pm$ 0.1
Crude fiber	23.67 $\pm$ 0.1
Ether extract	3.03 $\pm$ 0.1
Ash	10.48 $\pm$ 0.2
Nitrogen free extract	47.75 $\pm$ 0.2
Acid detergent fibre	43.56 $\pm$ 0.2
Neutral detergent fibre	61.23 $\pm$ 0.3
Metabolizable energy, Kcal/kg DM	2390.00

*Roadside grass predominating *Axonopus compressus*, *Panicum repens*, *Imperata cylindrica*, *Cynodon dactylon*, *Cynodon dactylon*, *Cyperus rotundus* species.

10.12. Dairy cow feeding trials

Experiment 1: Using plantain (*Plantago lanceolata*)

a. Animal ethics statement and location of the experiment

Experimental dairy cows handling, and milk and faeces samples collection were conducted in compliance with the Bangladesh Agricultural University Research System (BAURES) ethics. The duration of the study was 63 days from August 2020 to October 2020 while pandemic COVID-19 has been existed. The dairy cow feeding trial was performed at Boira, Bhaluka, Mymensingh. The humidity and ambient temperature of the region varied from 65-75 percent and 20-32°C, respectively during the study.

b. Experimental design, diet and management

A total of 12 cross Holstein Friesian breed (n=12) were randomly distributed into 4 dietary treatments (n=3 each) for 56 days plus 7 days adaption period. The animals were fed basal diet with plantain herb powder (PHP) at 0, 50, 100, and 150 gram/cow, and considering as PHP0, PHP50, PHP100, PHP150, respectively. The basal diet contains German grass, mustard oil cake, wheat bran, commercial feed, broken rice, DCP, and salt that was formulated in accordance with NRC-1985. The ingredients and chemical composition of basal were presented in Table 7. The

experiment was adopted a factorial design. Plantain herb was cultivated at the Shahjalal Animal Nutrition Field, BAU. Fresh plantain was harvested from experimental plots at the pre-blooming stage (~60 to 70 days), shade dried, and supplied to animals in addition to basal diet. Hand milking were performed twice a day at 0830 and 0430h. The plantain herb powder (PHP) supplementation was offered at a time in morning feeding at 0630h whereas feed was fed two times at 0630 and 0230h in day. The animals were cleaned twice a day just prior to milking. The clean and fresh water was supplied *ad-libitum*. Animals' data (milk yield, stage of lactation, days in milk production, and body weight) were recorded during and just prior to adaption period. Proper anthelmintic treatment was performed prior to 14days of the beginning of the experiment.

Table 7. Ingredients and nutrients composition of basal diet and plantain herb

Ingredients	Amounts	
	(g/kg of Fresh basis)	(g/kg of DM basis)
German grass	818.6	488.7
Broken rice	26.1	73.6
Mustard oil cake	30.6	88.5
Feed	26.1	72.4
Wheat bran	90.9	254.4
DCP	2.1	5.8
Salt	5.6	16.5
DM	31.21	88.00
Total	1000	1000
Analysed chemical composition		
Parameters	Basal diet (g/kg)	Plantain herb (g/kg)
OM	903.3	845.5
CP	149.3	150.7
CF	223.1	165.7
NDF	480	360
ADF	320	240.9
EE	47.1	275.13
Ash	96.7	154.5

c. Record keeping, sample collection and preparation

Milk yield and feed intake were recorded twice (morning and afternoon) a day for 56 days period while faecal samples were collected at the last 7 days of the experimental period. Milk and blood samples were on day 14, 28, 42 and 56 preserved at 4°C with potassium/sodium dichromate preservative (Sigma-Aldrich, St. Louis, USA) for further analysis. Besides, 50 mL milk sample were stored at -20°C for further analysis of milk fatty acids composition. For the determination of milk quality, the samples were freeze dried at -40°C to determine milk quality.

d. Milk samples analysis

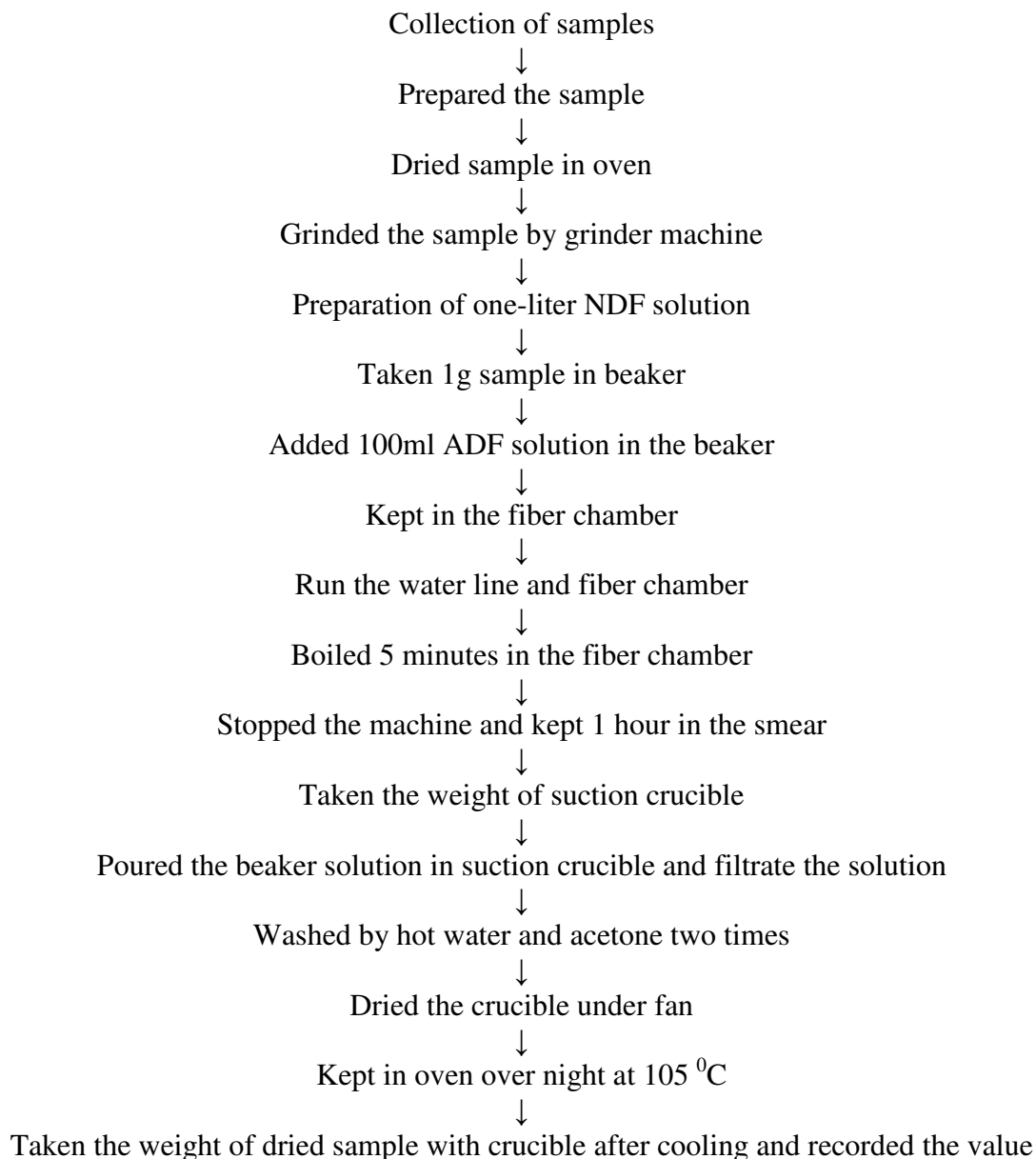
Milk composition (milk fat, protein, lactose, and minerals) were determined by lactoscan analyzer in the Dairy Science Lab, Bangladesh Agricultural University, Mymensingh. Free radical scavenging activity of milk samples was determined according to Nisha et al (2009) using

a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma Aldrich, Germany) assay. At first milk sample were freeze dried to determine the β -carotene level in milk. The total β -carotene content was calculated according to the equation: β -Carotene content (%) = $(A \cdot 5000) / (m \cdot 1480)$, where A is the absorbance measured at 421.0 nm, 5000 the dilution in ml, m the sample amount in g, and 1480 the A1/1 of β -carotene at 421.0 nm deduced from the A1/1-value of 2500 internationally established for the maximum of absorption of all-*E*- β -carotene in cyclohexane (De Ritter et al 1981). Milk samples were used for measuring the immunoglobulin (Ig), IgM, and IgA concentration in milk using bovine ELISA kits (Sigma-Aldrich, St. Louis, MO, USA). Milk fatty acid profile was determined at lipid chemistry lab of Bangladesh Council of Scientific and Industrial Research according to meat fatty acid profile.

e. Feed sample analysis

Proximate components (DM, CP, CF, EE, and Ash) were determined according the AOAC (2005) which was described at sheep feeding trail. The neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined according to the following protocols described by Goering and Van Soest (1970).

f. Determination of neutral detergent fibre (NDF)

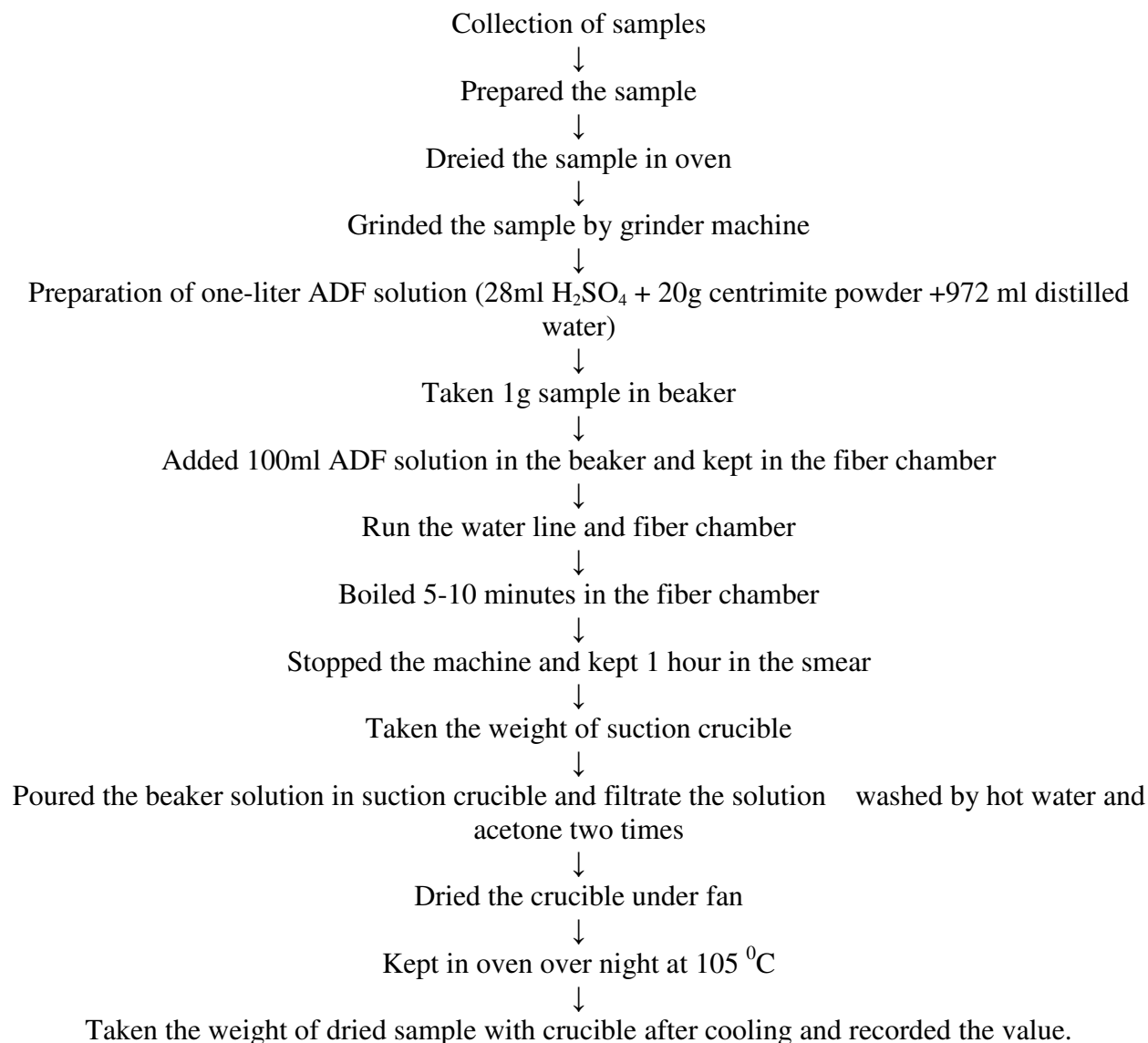


Flow diagm 5. Protocol for determination of NDF.

Calculation NDF % =

$$\frac{(Crucible+DM) wt - (Crucible+Ash) wt}{Sample wt * (\% \frac{DM}{100})} * 100 = \frac{(Crucible+DM) wt - (Crucible+Ash) wt}{Sample wt * (\% \frac{DM}{100})} * 100$$

g. Determination of acid detergent fibre (ADF)



Flow diagram 6. Protocol for determination of ADF.

Calculation % ADF =

$$\frac{(\text{Crucible} + \text{DM}) \text{ wt} - (\text{Crucible} + \text{Ash}) \text{ wt}}{\text{Sample wt} + \left(\% \frac{\text{DM}}{100}\right)} * 100 = \frac{(\text{Crucible} + \text{DM}) \text{ wt} - (\text{Crucible} + \text{Ash}) \text{ wt}}{\text{Sample wt} + \left(\% \frac{\text{DM}}{100}\right)} * 100$$

Statistical analysis : Results are presented as mean $\pm$ standard deviation. All data were subjected to One way ANOVA completely randomized design and the significance of differences among means was determined using Tukey's HSD test and the differences at $P <$

0.05 were considered statistically significant. All analyses were conducted by IBM SPSS 2011 (Version 20.0; IBM Corp., Armonk, New York, USA).

Experiment 2: using plantain (*Plantago lanceolata*) and lemongrass (*Cymbopogon citratus*)

The expt-2 was performed in the same farm with same procedures including, management practice, feeding regimen, sampling except number of animals which was 16 lactating dairy cows, period of study, during November to December 2020, and supplemental herbs and doses. The basal diet was based on German grass, mustard oil cake, wheat bran, commercial feed, broken rice, DCP, and salt that was formulated in accordance with NRC-1985. The ingredients and chemical composition of basal were presented in Table 8. The control diet was supplemented with 100 g plantain powder/day/cow (PT-D), 100g lemongrass powder/day/cow (LG-D); and 50 g plantain and 50 g lemon grass powder (PL-D). The experimental duration, sampling protocol and analytical techniques were similar to Dairy trial Expt-1 as stated above.

Table 8. Nutrient's digestibility of dairy cows offered herbal supplementation

Parameters	Diets				P-Value
	CL-D	PT-D	LG-D	PL-D	
DM	72.7	76.3	74.57	76.57	0.023
OM	74.54	78.16	76.49	77.66	0.050
CP	78.41	74.43	77.40	76.41	0.006
CF	65.5	71.75	68.00	69.95	0.009
-NDF	60.45	66.36	63.57	64.79	0.007
-ADF	51.78	56.75	53.48	56.11	0.094
EE	78.58	82.87	82.87	83.00	<0.001

CL-D: German grass, broken rice, wheat bran, mustard oil cake, DCP, and salt; PT-D: CL-D + 100 g plantain powder/day/cow, LG-D: CL-D+100g lemongrass powder/day/cow; PL-D: CL-D + 50 g plantain and 50 g lemon grass powder, SEM: standard error of mean, P<0.05 significant

a. Mouse model validation trial

Research animals and management : The experimental Swiss albino mice (*Mus musculus*), weighing 26±4 g and 42 days aged were collected from Jahangir Nagar University, Savar, Dhaka having apparently good health and devoid of any external deformities certified by the registered veterinarian from BAU. The mice were placed in cage made of galvanized iron sheet having 5 cm thick saw dust litter. Mice were reared under normal condition of temperature (23-25°C) and humidity with a provision of feed and water *ad libitum*. FA solution was prepared from stock paraformaldehyde powder (Merck, Darmstadt, Germany) by thermal depolymerisation. The study was conducted from February to April at Feed Safety and Phyto-Nutrition lab under the Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh, Bangladesh. All mice were handled according to the animal care in compliance with the Bangladesh Agricultural University Research System (BAURES) on the care and use of laboratory animals.

b. Experimental Design

For experimental purpose, the mice were randomly allocated into five groups like: control (C), control with acteoside standard at the 5mg/kg BW/day (A5), control with fresh plantain at 5 (P5), 10 (P10) and 20 (P20) mg/kg BW/day. Each group had 4 mice. Mouse were offered formulated diet as control. The acteoside standard and fresh plantain extract were supplemented through drinking water.

c. Serum biochemical analysis

Mice were anaesthetized with formalin during sacrifice. Thoracotomy was performed. Blood was directly collected from heart and kept in falcon tube. Serum was separated from unclothed blood by centrifuge at 3410 rpm for 15 minutes and again for 10 minutes. Then supernatant was collected in eppendorf tube by micro-pipette and stored in refrigerator at -20°C until use for biochemical test.

Serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) concentrations were carried out using different commercial enzymatic assay kits (Sigma Aldrich and Bio-vision, Germany, and US) in a biochemical analyzer (Urit810; URIT Medical Electronic Group Co., Ltd., Guangxi, China) according to the method of the commercial kit procedure. AST: ALT was calculated using the value of AST divided by value of ALT.

For serum superoxide dismutase (SOD) determination, 20 µL sample was mixed with 200 µL water-soluble tetrazolium salt solution and 20 µL enzyme working solution. The mixture was kept at 37°C for 20 minutes and after that took absorbance at 450 nm using a microplate reader (EL 10A; BIOBASE, China) as instruction provided in Cat. No.19160, Sigma-Aldrich, Germany. For glutathione peroxidase (GPx) determination specific ELISA kit (Cat. No. 354102, Sigma-Aldrich, Germany) was used, and exactly 20 µL sample was mixed with 50 µL assay buffer, 50 µL co-substrate mixture and, 50 µL NADPH according to manufacturer instruction. Finally, the absorbance was taken at 356nm wavelength. Similar to SOD and GPx, catalase was determined using an assay kit according to the manufacturer's protocol (Cat. No. 100, Sigma-Aldrich, Germany).

d. Histopathology and immunohistochemistry

Digestive organs (Stomach, duodenum, jejunum, ileum, ceacum, colon, rectum, liver, , spleen) and immune organ bursa tissues from were collected and immediately fixed in 10% neutral buffered formalin solution. Deparaffinized sections were stained with hematoxylin and eosin (HE) for morphological observations, and with Azan–Mallory stain for detecting collagen deposition. PLP fixed deparaffinized sections were immunostained with (monoclonal or polyclonal) primary antibodies and horseradish peroxidase-conjugated secondary antibody (Histofine simplestain MAX-PO, Nichirei, Tokyo, Japan). Tissue sections were immunostained by the Histostainer (Histofine, Nichirei Bioscience Inc., Tokyo, Japan). Positive reactions were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB substrate kit, Vector Laboratories, Burlingame, CA, USA), and the sections were lightly counterstained with

hematoxylin. As negative controls, tissue sections were treated with mouse or rabbit non-immunized serum instead of the primary antibody.

Statistical analysis : Results are presented as mean. All data were subjected to one-way ANOVA, and the significance of differences among means was determined using Tukey's HSD test and the differences at $P < 0.05$ were considered statistically significant. All analyses were conducted by IBM SPSS 2011 (Version 20.0; IBM Corp., Armonk, New York, USA).

11. Results and discussion (*Combined*)

11.1. Baseline survey on daily calorie intake of street children in capital city, Dhaka

A total of 384 street children were subjected to interview for baseline survey on their present calorie (kcal) intake per day. RDI for 4-12 yrs. boys and girls are ranged from 1303-2200 kcal and 1202-2070 kcal respectively. Survey data revealed that 27-59% gap or deficiency of RDI prevailed in Boys population (Table 1) and 28-56% gap of RDI for girls population (Table 9). We have predicted while our ongoing baseline survey that if we can produce to supplement 500 kcal by 100 g serving once a day then minimization of RDI gap can be 38-100% and 56 -100% for boys and girls of 4-12 yrs. Respectively (Table 9 and 10). Z-score can identify and pinpoint deterioration in weight and height of children in reference to the children of same age and sex. Z-score is more accurate and reliable way of presenting prevalence data in population level surveys. In Z score analysis considering Weight for Age condition, less than -2σ score is classified as underweight, score between -2σ to -3σ classified as moderate underweight and more than -3σ score classified as severe underweight. On the other hand, in Z score analysis considering Height for Age condition, less than -2σ score is classified as stunting, score between -2σ to -3σ classified as moderate stunting and more than -3σ score classified as severe stunting [18]. Data revealed that both boys and girls for *Weight for Age and Height* for age were less than -2σ score which classified as underweight and stunting population of 4-12 yrs. in our current baseline survey for street children respectively (Fig 7 & 8). In our statistical data analysis, less than 5th percentile were used to define shortness and low weight-for-length of our target population for both Boys and Girls of 4-12 yrs.

Table 9. Baseline survey on daily dietary intake of street children (age 4-12) for boys

	Age	RDI	Observed	Observed	RDI	Gap	Gap	EDRB	EDRB	Gap	Predicted
		In	Average	ved	%	In	% of	Kcal	Provide	% of	Kcal gap
		Kcal	In Kcal	Ranges in Kcal	Intake	Kcal	RDI	Per 100g serving	RDI %	RDI % Remain	Minimization of RDI%
Male	4 (n=13)	1303	955	600-1100	73	348	27	500	38	-11	100
	5 (n=11)	1362	865	630-1210	63	497	37	500	37	0	100
	6 (n=14)	1403	852	670-1180	61	551	39	500	36	3	92
	7 (n=21)	1507	994	720-1187	66	513	34	500	33	1	97
n=243	8 (n=20)	1624	919	620-1170	57	705	43	500	30	13	70
	9 (n=35)	1750	865	620-1250	49	885	51	500	28	23	55
	10 (n=32)	1890	877	670-1300	46	1013	54	500	26	28	48
	11 (n=40)	2038	866	620-1400	42	1172	58	500	24	34	41
	12 (n=57)	2200	893	650-1280	41	1307	59	500	23	36	38
EDRB: Energy dense rice biscuit; ED: Energy Density; RDI: Recommended dietary intake											

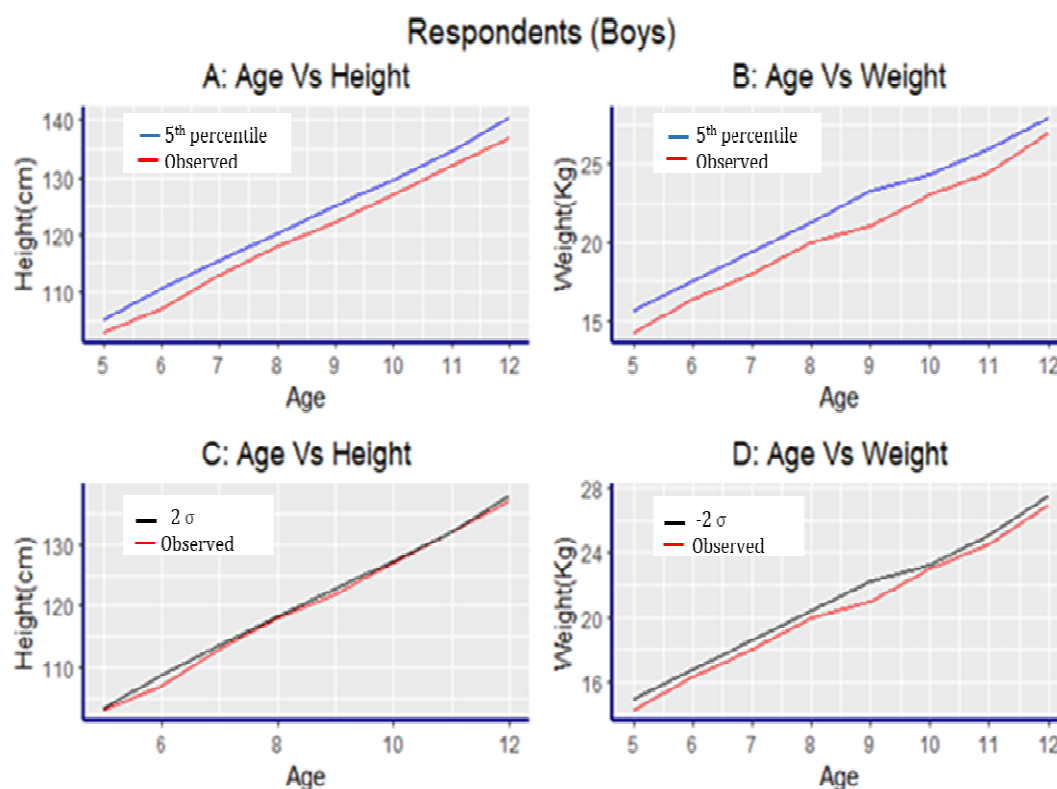
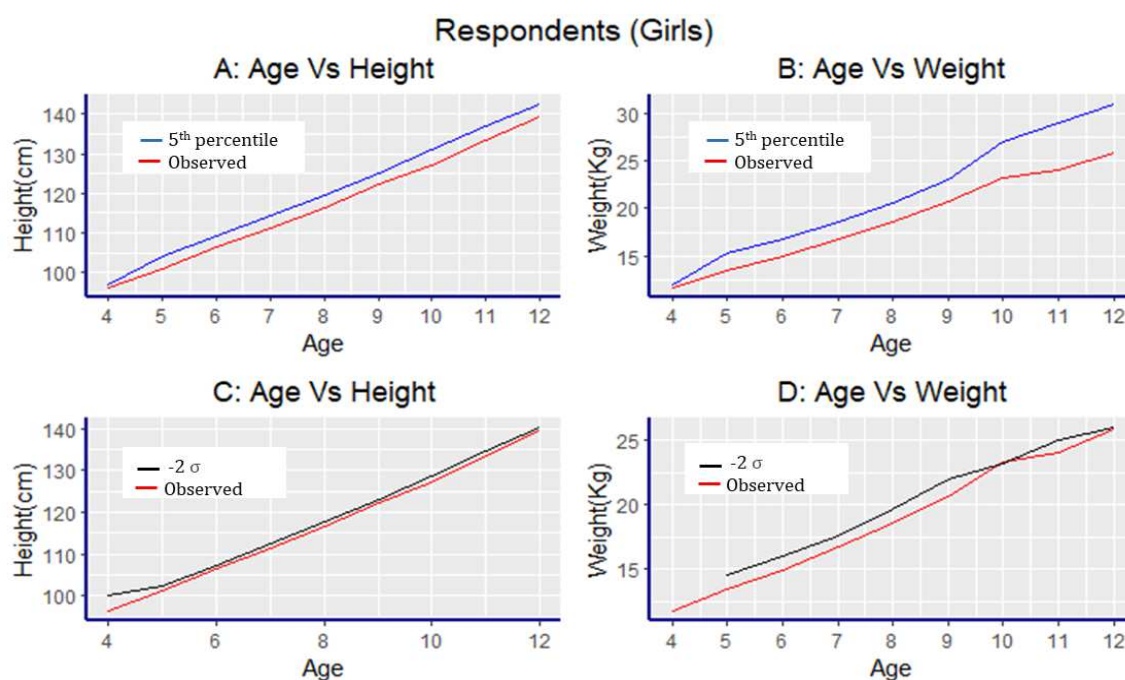
**Fig 7.** Percentile and Z-Score of Boy's population (n=243) Height for Age and Weight for Age.

Table 10. Baseline survey on daily dietary intake of street children (age 4-12) for girls

Female	Age	RDI in	Observed	Observed	RDI	Gap	Gap	EDRB/EDRC	EDRB/EDRC	Gap %	Predicted Kcal gap
			(Average) data	Data	%	In	% of	Kcal	Provide	of	Minimization
		Kcal	In Kcal	Ranges in Kcal	Intake	Kcal	RDI	Per 100g serving	RDI%	RDI% Remain	of RDI%
Female	4 (n=12)	1202	898	625-1110	72	346	28	500	41	-14	100
	5 (n=19)	1244	850	700-1300	68	352	32	500	41	-14	100
	6 (n=11)	1300	769	658-1020	59	531	41	500	38	3	93
	7 (n=28)	1403	884	620-1180	63	519	35	500	37	-2	100
	8 (n=11)	1502	1002	820-1210	67	500	33	500	33	0	100
n=141	9 (n=13)	1638	767	672- 970	47	871	53	500	30	23	56
	10 (n=15)	1777	931	670-1170	52	846	48	500	28	29	60
	11 (n=21)	1942	863	650-1025	44	1079	56	500	26	30	54
	12 (n=11)	2070	930	850-1270	45	1140	55	500	24	31	56
EDRB: Energy dense rice biscuit; ED: Energy Density; RDI: Recommended dietary intake											

**Fig 8.** Percentile and Z-Score of Boy's population (n=141) Height for Age and Weight for Age.

11.2. Baseline survey of existing fried chips products from processing to marketing in selected project locations of Bangladesh

In Bangladesh, potato chips and extruded products (crackers) are available commonly in urban areas. During the survey with the help of pre-tested questionnaires (producer based and hawker based), the complete data from producer to consumer was collected from the selected locations. The baseline information was collected from seven districts namely Dhaka, Gazipur, Tangail, Mymensingh, Bogura, Munshigonj and Jashore. The information was accumulated from the respondent of different locations (such as school, park, highways, bus, train etc.). Selling pattern (selling area, retail price, profit, selling time, consumer level etc.) and the processing pattern with their frying practices such as frying technique, frying oil type, frying temperature & time, raw materials, packaging, storage etc. were also collected. The collected baseline general information was analysed and shown in Table 11. It was observed that most of the Hawkers were aged between 25-39 years. However, in Gazipur and Mymensingh region some hawkers were below 17 years old. In case of producers, the age groups were above 40 years and 25-39 years old. In Munshigonj 100% producers were in the age group of 25–39 years and in Bogura 80% were age group above 40 years where most of them were illiterate. Similar trends were noted in Munshigonj where the producers' education level is under class V. Due to illiteracy, they had no knowledge about the product quality maintenance during processing to marketing.

In Table 12, it was observed that the Hawker of Bogura, Jashore and Munshigonj processors/producers were Hawkers and they prepared the product and distributed the products to the market or directly involved in sale. It might be happened because of being a huge quantity of production in those areas. However, the Hawkers of Tangail area collected almost all potato chips from the wholesalers. Similar information was noticed in Gazipur, Dhaka and Mymensingh areas. In Jashore, Munshigonj and Dhaka hawkers (60–100%) earned above 500 Taka profit per day to sell potato chips and Gazipur (40%) and Mymensingh (50%) hawkers had profit 350-500 Taka per day. Medium profit/day (201-349 Taka) was achieved by both in Bogura and Tangail Hawkers (60%). Both in Bogura and Munshigonj Hawkers had no idea about the product healthiness but most of Dhaka and Jashore Hawkers were known about potato chips processing in hygienic condition because of maintaining product quality and safety issue. In the study area most of the potato chips consumers were all ages except in Jashore was observed 80% child preferred potato chips (Table 12).

The result presented in Table 13 shown 100% producers of Bogura, Mymensingh, Jashore and Munshigonj used deep frying technique and more than 50% small processors prepared potato chips into deep oil frying with soya bean oil. Some processor used mixed oil as soybean and palm oil together. Most of respondents (>80%) of those locations changed oil during frying of potato chips when frying oil turned into cloudy and 17-20% respondent changed frying oil when perceived smelt burn during frying. Hundred percent respondents did not know frying oil temperature during processing of fried chips. Frying temperature influences product quality with safety assurance. Most of them determined the oil quality by observing oil color during frying where oil temperature is the most concerning factor to reduce the acrylamide content in the fried products. Product fried into deep oil at higher temperature may be a possibility to contain acrylamide substance, which is carcinogenic element (Pedreschi and Moyano, 2005). Most of the producers of potato chips processing counted frying time as per product crispiness except the

study area of Mymensingh. In that area, product color change is an important indicator to evaluate the frying completion time.

Table 11. General Information for the respondent of hawkers and producers during baseline survey

Hawkers based (%)								Producers based (%)			
Particular	A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	B ₁	B ₂	B ₃	B ₄
A. Age Group											
Below 17 year	53.33	-	-	37.50	-	-	-	-	-	-	-
18-24 year	6.67	20.00	40.00	25.00	-	-	20.00	-	-	-	-
25-39 year	40.00	60.00	40.00	12.50	20.00	80.00	80.00	60.00	20.00	60	100.00
40 above	-	20.00	20.00	25.00	80.00	20.00	-	40.00	80.00	40	-
B. Education Level											
Illiterate	73.33	30.00	60.00	62.50	80.00	20.00	100	60.00	80.00	60.00	66.66
Class I-V	26.67	30.00	40.00	37.50	20.00	20.00	-	40.00	20.00	40.00	33.33
Class VI-X	-	40.00	-	-	-	60.00	-	-	-	-	-
SSC	-	-	-	-	-	-	-	-	-	-	-
HSC	-	-	-	-	-	-	-	-	-	-	-

Note: Hawkerc based (A₁-Gazipur; A₂-Dhaka; A₃-Tangail; A₄-Mymensingh; A₅-Bogura; A₆-Jashore; A₇-Munshigonj; Producers based (B₁-Mymensingh; B₂-Bogura; B₃-Jashore; B₄-Munshigonj)

Table 12. Hawker based data collection of different fried chips from selected project locations

Study area	Product collection (%)		Profit/day (%)				Knowledge about healthiness (%)			Consumer (%)		
	Own making	Wholesaler	Low	Medium	High	Highest	Healthy	Unhealthy	No Idea	Child	Young	All Age
Gazipur	13.33	86.67	26.67	20.00	40.00	13.33	26.67	6.66	66.67	20.00	-	80.00
Dhaka	30.00	70.00	-	20.00	20.00	60.00	70.00	-	30.00	40.00	-	60.00
Tangail	-	100.00	20.00	60.00	20.00	-	20.00	-	80.00	20.00	-	80.00
Mymensingh	37.50	62.50	-	25.00	50.00	25.00	25.00	-	75.00	25.00	-	75.00
Bogura	100.00	-	20.00	60.00	20.00	-	-	-	100.00	-	-	100.00
Jashore	100.00	-	-	-	-	100.00	60.00	-	40.00	80.00	-	20.00
Munsh-igonj	100.00	-	-	-	20.00	80.00	-	-	100.00	20.00	20.00	60.00

Note: Profit ranges category: Low=150-200 Tk/day; Medium=201-349 Tk/day; High=350-500 Tk/day; Highest=Above 500 Tk/day

Table 13. Producer based data collection of different fried chips from selected project locations

Study area	FT used by small processors (%)		Frying oil used in small processors (%)			Respond on oil temperature (%)		Respond on oil change condition (%)		Respond on frying completion time (%)	
	DF	VF	Soya-bean oil	Palm oil	Mixed oil	Known	Unknown	Cloudy	Smelt burn	Color change	Crispiness
Bogura	100.00	-	80	20.00	-	-	100.00	80.00	20.00	20.00	80.00
Mymensingh	100.00	-	80	-	20.00	-	100.00	100.00	-	60.00	40.00
Jashore	100.00	-	50	-	50.00	-	100.00	83.33	16.66	33.33	66.66
Munsh-igonj	100.00	-	100	-	-	-	100.00	80.00	20.00	20.00	80.00

Note: FT=Frying technique; DF=Deep frying; VF=Vacuum frying



Plate 12. Baseline information with sample collection from Agargaon, Dhaka

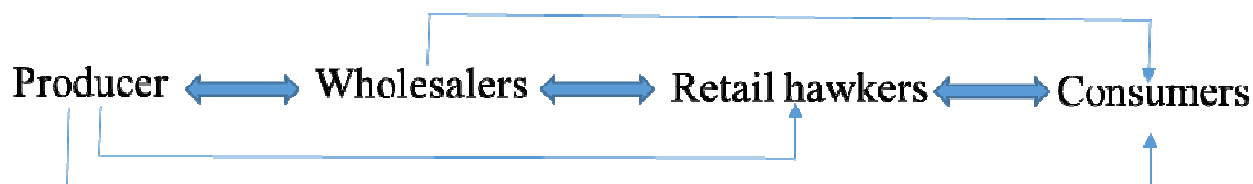


Plate 13. Baseline information with sample collection from High way Road, Tangail



Plate 14. Baseline information with sample collection from Bogura

Marketing and distribution channel In Bangladesh, locally available potato chips distribution from producers to consumers comprises of producers, wholesaler, retailers/hawkers and consumers. In this product distribution structure, the processors/producers are most powerful as the entire distribution channel is dependent on them. Sometimes producers work as wholesalers and even they directly distribute the products to the consumers. From the survey, it is observed that in the direct distribution system of the product from producers to consumers they get the highest profit comparatively (Table 12). Retail hawkers also collect the products from processors and distributes to the consumers. However, they get the limited profit. Generally, middleman adds the products distribution cost and lowers the profits. As the locally available potato chips in Bangladesh are shorter shelf life generally used to market within a shortage period of storage (within 2-3 days). In Dhaka and Gazipur, retailing hawkers to consumer's product distribution system is commonly practiced. However, except Dhaka and Gazipur other processors as well as wholesalers directly distribute the potato chips directly to the consumers. The marketing channel of the locally available potato chips in Bangladesh is given below:



Flow diagram 7. Locally available potato chips marketing and distribution channel.

11.3. Formulation of low cost, nutritious and safe rice-based food

Since rice flour does not have gluten protein unlike wheat, so making dough or batter was seemed difficult to get appropriate texture in reality. We had tried to overcome the problem with few measurements such as follows, i. Un-parboiled milled rice was treated for one hour soaking in water at room temperature before grinding into flour, ii. Parboiled rice was used for rice-based bakery products subjected to low Glycemic rice products only and in other case un-parboiled milled rice will be uses for bakery purposes, iii, Both low apparent amylose content (AAC) and gelatinization temperature (GT) rice flour were considered suitable parameters for rice-based bakery products., iv, Rice flour parameters such as oil absorption capacity (OAC), water absorption capacity (WAC) and forming activity etc. were also considered at flour quality selection, v. Sheaving flour to attain unique flour size was a necessary step and sagu, casava and aloevera can potentially be used as thickening, stabilizing, suspending, and binding agent. Ingredients of ERDB and EDRC were rice flour, sugar, skimmed milk, egg, vanilla essence, grinded sagu powder as lubricating agent, butter and RBO. Bangladesh Standards and Testing Institute (BSTI) is the prime standardizing authority in Bangladesh for food quality control. Since BSTI did not have currently rice standards but wheat flour only so, we formulated rice-based biscuit with reference of high protein wheat biscuit prescribed by BSTI and tried to fortified fat content which formulated first time in Bangladesh. Our energy dense rice biscuit (EDRB) and cake (EDRC) had energy density ranges from 5.0-5.15 which means per 100 g serving EDRB and EDRC can generate 515 and 500 kcal respectively (Table 14 & 15). According to BSTI minerals such as Zn, Fe, Ca, Phos. and heavy metals such as As, Pb, Cd and Ni were supposed to be quantify and check the presence in rice-based products respectively. We quantified all required minerals and checked the presence of heavy metals in our products (Table 14 & 15). For our products we used, 57-59% carbohydrate from rice flour and sagu powder together, then 25 to 27 % fat from Rice bran oil and butter together, then 10 to 10.6% protein from milk, egg together (Table 13 & Table 14). Proportionate use of sagu powder mixed with rice flour gave good bakery texture for rice biscuit and cake as sagu powder served as binding agent. Rice-based formulated diets for children with moderate to acute malnutrition must have some important characteristics including high content of micronutrients, especially growth (Type II) nutrients, high energy density (defined as the ratio of energy per weight of food), adequate high protein and fat content, low content of anti-nutrients, low risk of contamination, acceptable taste and texture, culturally acceptable, easy to prepare, affordable and available. High energy density rice is most desirable for children with wasting, as they have an increased energy need for catch-up growth. The most important factor influencing energy density is the fat content, as the energy density of fat (9 Kcalg^{-1}) according to the Atwater factors is more than double that of protein and carbohydrate (4 Kcalg^{-1}). A major factor constraining accessibility of poor is price volatility. As the poor spend a relatively larger proportion of their income in basic staples, a sudden spike in price is equivalent to reduction in their real incomes. This will constrain their ability to acquire adequate quantity of staples. Considering this fact into account, our formulated products will not cost more than \$0.10 USD or 8.5 BDT for 100 g serving which supposed to be cost effective.

Table 14. Proximate analysis of EDRB (Energy dense rice biscuit)

Proximate components	Amount in g	Kcal ⁻¹ g	Estimated Kcal	Minerals	Amount ⁻¹ 100 g
Carbohydrate	57	4	228	Zn	2.50 mg
Fat	27	9	243	Fe	0.71 mg
Protein	10.6	4	42.4	Ca	3.80 mg
Dietary Fiber	1	2	2	Phos	125 mg
Ash	0.8	0	0	As	<0.1 ppm
Moisture	3.6	0	0	Pb	<0.1 ppm
Net weight	100	-	515.4	Cd	<0.1 ppm
Energy Density (ED)	5.2	-	515 Kcal	Ni	<0.1 ppm

Table 15. Proximate analysis of EDRC (Energy dense rice cake)

Proximate components	Amount in g	Kcal ⁻¹ g	Estimated Kcal	Minerals	Amount ⁻¹ 100 g
Carbohydrate	59	4	236	Zn	2.30 mg
Fat	25	9	225	Fe	0.51 mg
Protein	10	4	40	Ca	3.60 mg
Dietary Fiber	0.5	2	1	Phos	115 mg
Ash	0.5	0	0	As	<0.1 ppm
Moisture	5.0	0	0	Pb	<0.1 ppm
Net weight	100	-	502	Cd	<0.1 ppm
Energy Density (ED)	5.02	-	502 Kcal	Ni	<0.1 ppm

11.3.1 Impact evaluation study of rice based formulated food on street children

Our population target is children at age of 4 to 12 years and their expected weight ranges from 16.71 to 34.38 kg for boys and 16.0 to 37.0 kg for girls at Bangladesh population standard including both sexes (from 4 years to 12 yrs. of age) ranges from 1303-2200 and 1202-2070 Kcal/day. According to FAO for Bangladeshi population [7,8] which requires 78 to 64 Kcal/kg/day for boys and 75 to 56 kcal/kg/day for girls respectively (from 4 years to 12 yrs. of age) as FAO reference (8). A total of 52 respondents including 32 testing and 35 controls samples of both sexes were kept in impact study of formulated food (Rice based cake or biscuit) on street children. We have set up a free one-hour schooling at our project field at Uttara near the Turag river side where informal education was given for 4 months. We tried to monitor that the serving amount of 100 g equivalent rice cake were properly intake by selected 32 volunteers or respondents at daily basis. In order to monitor our feeding activities, we have to set up this type of field school temporarily. Blood samples were taken at day 0 as initial and 100 g of serving per day formulated food (Rice based cake or biscuit) were given to 32 testing samples every day for 4 months' time without hampering their normal food intake. On the other-hand controls were not feed the similar amount of 100 g of serving per day formulated food (Rice based cake or biscuit) but keeping them on their usual intake of food. Control group for 20 street children were subjected to draw the blood samples as initial too. At the end of 4 months' time blood samples from both samples and controls were taken for comparison of blood parameters

specially CBC (Complete Blood Count), Hemoglobin, CRP and Prealbumin. Blood parameters related to malnutrition such as CBC (Complete Blood Count), Hemoglobin, CRP and Prealbumin were collected at both the starting and the end time of the impact survey of selected 52 respondents including testing and control samples.

In impact study at day 0, fifty-two (52) respondents including 32 testing samples and 20 control samples were subjected to draw blood samples for estimating several malnutrition related blood parameters such as CBC (Complete Blood Count), Hemoglobin, CRP and Prealbumin were examined. These respondents also subjected for their daily Calorie intake baseline survey at day 0. From that onwards, a supplementary energy dense rice cake (EDRC) having ED 5.0 was distributed among 32 testing samples for 120 days or 4 months duration without interfering their regular food intake. Since testing samples were given 500 kcal supplements so at the end of the 4-month time their daily energy intake found higher than control group which reflects in overall improvement in blood parameters such as increased Hb%, Total White cell count (TWC), Red blood cell (RBC), Prealbumin and decreased CRP protein. Our data revealed that the Deficiency of RDI% in our population street children for respective age group were improved by 70-100% at day 120 for taking supplementary rice-based food once a day (Table 16). Before starting the impact study, our target samplings for both control (n=20) and treatment groups (n=32) were checked statistically by Independent t test at day 0 ($\alpha = 0.05$) since there was no mean difference between control and treatment group at day 0 for hemoglobin, TWC, RBC, CRP and Prealbumin (Table 17). Null hypothesis for the mean differences between the before and after treatment at day 120 for hemoglobin, TWC, RBC, CRP and Prealbumin were found significant by Paired t test ($\alpha=0.05$) (Table 18). Finally, Null hypothesis for measuring mean difference between control and treatment group at day 120 for several blood parameters such as hemoglobin, TWC, RBC, CRP and Prealbumin were found significant by Independent t-test $\alpha = 0.05$ (Table 19). All statistical analysis clearly indicate that there was a significant improvement in treatment or testing group compared to control group. EDRC can potentially be used in school feeding nutrition program and disaster management including refugee crisis, flood or cyclone related emergency relief operation program to mitigate malnutrition and emergency response of energy dense dry food as supplements. Thus we can attain SDG goal 2.0 (Zero hunger) specially 2.1 goal which deals with a vision to end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round.

Table 16. Impact study of EDRC on street children (age 4-12 yrs. for 14 boys and 18 girls)

Name	Age	Gender	Daily Energy at 0 day (Observed) Kcal/day	Age based RDI Kcal/day	Deficiency %	EDRC Kcal/day	Daily Energy at 120 day (Observed) Kcal/day	Deficiency %	Deficiency status improved
Rohita	5	Female	880	1202	26.8	500.0	1380	-14.8	100
Hasan	5	Male	1160	1362	14.8	500.0	1660	-21.9	100
Siam	6	Male	1180	1403	15.9	500.0	1680	-19.7	100
Samia	6	Female	1290	1300	0.8	500.0	1790	-37.7	100
Lamia	6	Female	1210	1300	6.9	500.0	1710	-31.5	100
Fahima	6	Female	1240	1300	4.6	500.0	1740	-33.8	100
Mim	6	Female	1230	1300	5.4	500.0	1730	-33.1	100
Shohid	6	male	1130	1403	19.5	500.0	1630	-16.2	100
Itila Akter	6	Female	1030	1300	20.8	500.0	1530	-17.7	100
Yeasin	7	Male	1280	1507	15.1	500.0	1780	-18.1	100
Sakib	7	Male	1280	1507	15.1	500.0	1780	-18.1	100
Jhuma	8	Female	1280	1502	14.8	500.0	1780	-18.5	100
Tinni Akter	8	Female	1080	1502	28.1	500.0	1580	-5.2	100
Mahfuj	8	Male	1340	1624	17.5	500.0	1840	-13.3	100
Susmita	8	Female	1230	1502	18.1	500.0	1730	-15.2	100
Maria	8	Female	1230	1502	18.1	500.0	1730	-15.2	100
Mehedi Hasan	9	Male	1080	1750	38.3	500.0	1580	9.7	92.3
Irin Akter Shila	9	Female	1340	1638	18.2	500.0	1840	-12.3	100
Miraz	10	Male	1410	1890	25.4	500.0	1910	-1.1	100
Akash	10	Male	1310	1890	30.7	500.0	1810	4.2	95.8
Konika	10	Female	1030	1777	42.0	500.0	1530	13.9	86.1
Rbbi Miah	11	Male	1330	2038	34.7	500.0	1830	10.2	89.8
Md. Yakub	11	Male	1280	2038	37.2	500.0	1780	12.7	87.3
Md. Sakib Miah	11	Male	930	2038	54.4	500.0	1430	29.8	70.2
Md. Jaman	11	Male	1230	2038	39.6	500.0	1730	15.1	84.9
Kiran Miah	11	Male	1030	2038	49.5	500.0	1530	24.9	75.1
Farzana	11	Female	1210	1942	37.7	500.0	1710	11.9	88.1
Mithila	11	Female	930	1942	52.1	500.0	1430	26.4	73.6
Sharmin	11	Female	1230	1942	36.7	500.0	1730	10.9	89.1
Nishita	11	Female	1080	1942	44.4	500.0	1580	18.6	81.4
Choity Akter	11	Female	1130	1942	41.8	500.0	1630	16.1	83.9
Fatima Akter	12	Female	1180	2070	43.0	500.0	1680	18.8	81.2

Table 17. Null hypothesis for selecting samples for control and treatment group at day 0

Null Hypothesis	Male		Female	
	P value	Decision	P value	Decision
There is no mean difference between control and treatment group at day 0 of hemoglobin	0.6331	Not rejected	0.7155	Not rejected
TWC	0.7534	Not rejected	0.7939	Not rejected
CRP	0.6779	Not rejected	0.6197	Not rejected
Prealbumin	0.899	Not rejected	0.6777	Not rejected
Independent t test at day 0 ($\alpha = 0.05$)				

Table 18. Null hypothesis for the mean differences between the pre and post treatment at day 120

Null Hypothesis	Male		Female	
	P value	Decision	P value	Decision
The mean differences between the before and after treatment of Hemoglobin readings equals 0	7.781e-05	Reject the null hypothesis	5.059e-07	Reject the null hypothesis
The mean of differences between the before and after treatment of CRP readings equals 0	0.0260	Reject the null hypothesis	0.0007	Reject the null hypothesis
The mean of differences between the before and after treatment of Prealbumin readings equals 0	5.34e-08	Reject the null hypothesis	1.497e-08	Reject the null hypothesis
The mean of differences between the before and after treatment of TWC readings equals 0	0.0016	Reject the null hypothesis	0.0007	Reject the null hypothesis
Paired t test ($\alpha = 0.05$)				

Table 19. Null hypothesis for measuring mean difference between for control and treatment group at day 120

Null Hypothesis	Male		Femle	
	P value	Decision	P value	Decision
There is no mean difference between control and treatment group at 120 days of Hemoglobin	0.0004	Reject the null hypothesis	0.0307	Reject the null hypothesis
There is no mean difference between control and treatment group at 120 days of TWC	0.0411	Reject the null hypothesis	0.0480	Reject the null hypothesis
There is no mean difference between control and treatment group at 120 days of CRP	0.0078	Reject the null hypothesis	0.0003	Reject the null hypothesis
There is no mean difference between control and treatment group at 120 days of Prealbumin	0.0419	Reject the null hypothesis	0.0410	Reject the null hypothesis
Independent t test at day 120 ($\alpha = 0.05$)				

11.3.2. Specifications of the designed and fabricated vacuum frying machine

According to design considerations and Solidworks drawings, a low-cost small-scale vacuum frying machine was developed. The schematic views of the machine of the frying machine are shown in Fig 9. Specifications of the major parts of the fabricated vacuum frying machine are shown in Table 20. The developed machine was constructed with low-cost parts and easy to operate. The spare parts of the machine are available and affordable to exchange. The small size frying machine is feasible to move from one place to another with the nylon wheel attached with the base.

Table 20. Specification of the vacuum frying machine

Sl. No.	Parameter	Dimension/Specification
01	Dimension of chassis	(860×390×40) mm
02	Dimension of control panel	(300×300×180) mm
03	Dimension of condenser	(520×165) mm
04	Size of frying tank	(360×412) mm
05	Power of the vacuum pump	1 Hp
06	Vacuum pump type	Two stage vacuum pump
07	Ultimate vacuum	-760 mmHg
08	Size of sample holding basket	(300×210) mm
09	Capacity of the heater (02 no.)	4000 watts, 220V, AC
10	Capacity of oil chamber	15 - 20 L
11	Capacity of raw chips product	800-1000g/batch
12	Range of frying temperature	0 – 400 °C

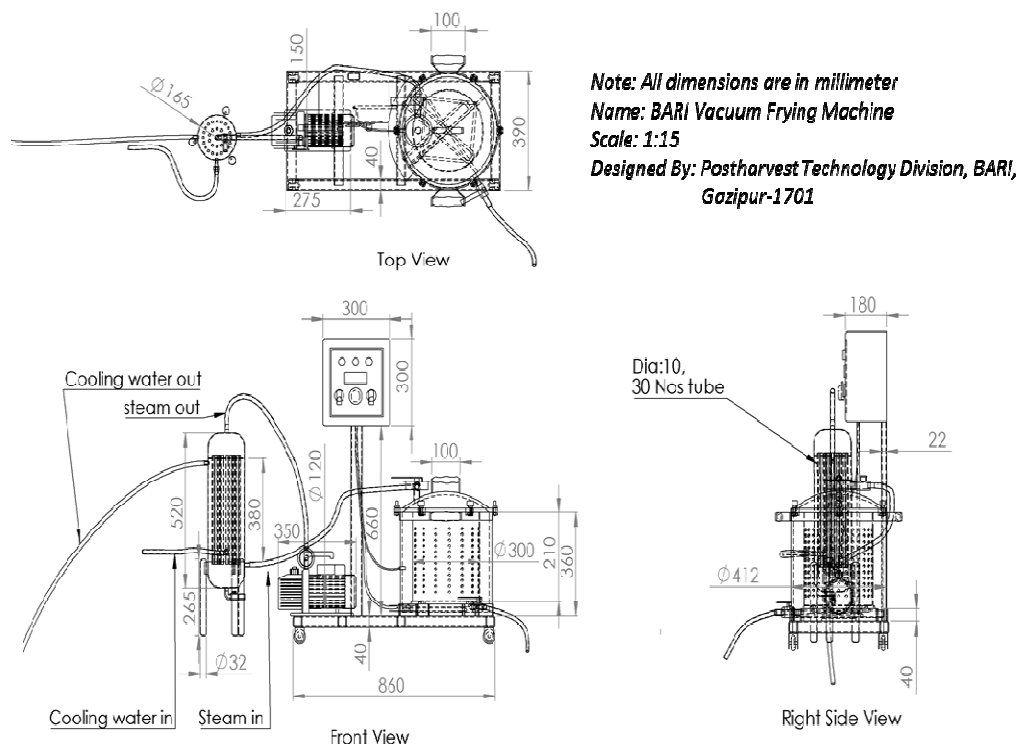


Fig 9. Schematic view of the simplified design BARI Vacuum frying machine.

11.3.3. Working principle of the vacuum frying machine

Vacuum frying is differential to conventional frying, as it is carried out at lower temperature and pressure with minimal processing of the produces. Due to lower pressure in the frying chamber the boiling point of water lowers, and the moisture of the raw sample starts to evaporate rapidly and products turns into chips form. In absence of air in contact with the products there is no chance to cause Maillard reaction, for that reasons products color natural color retains. Control panel is used to set the optimum frying temperature of the frying oil. After the temperature rise the pre-treated raw materials are loaded in the sample holder basket and the kept in the frying chamber and the vessel lid is closed tightly. The cooled water inlet line is needed to open in the condenser. Vacuum pump is switched on and countdown the frying time and check the vacuum pressure also. After optimum frying time the products are unloaded by opening the two-way gate valve in the frying chamber to release the pressure. A completely fried product becomes crispy and attractive with good organoleptic and nutritional properties of the fried products. It is an efficient method to produce fruits and vegetables snacks with the necessary degree of dehydration without excessive darkening or scorching (Plate 15).

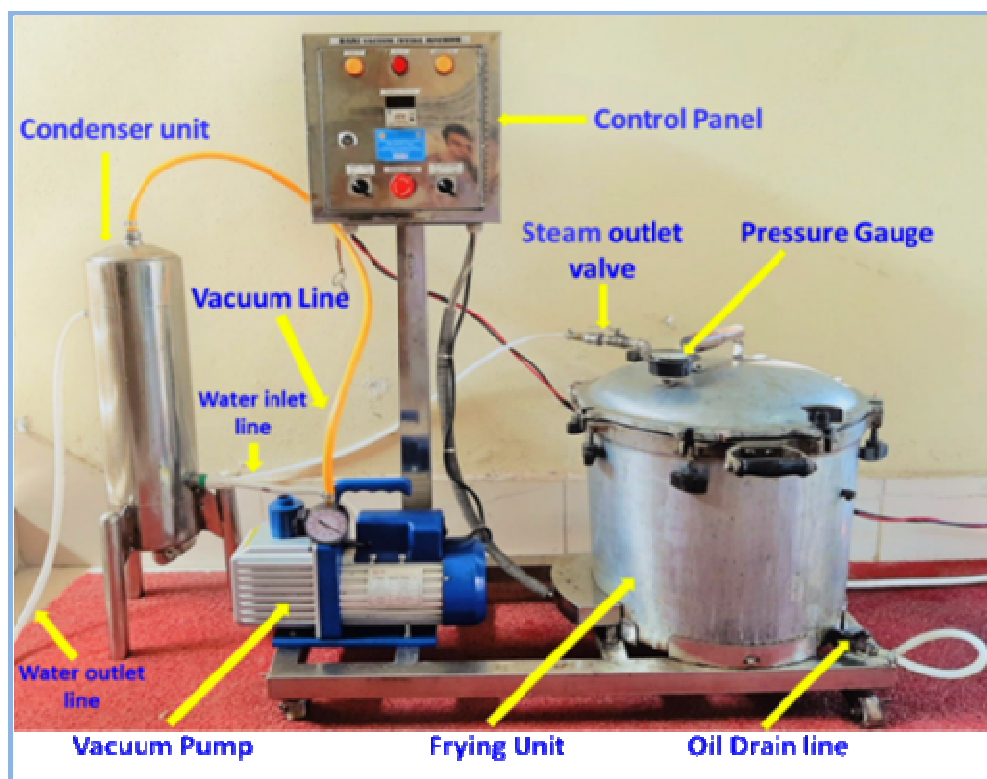


Plate 15. BARI developed low-cost Vacuum frying machine.

11.3.4 Specifications of the designed and fabricated de-oiling machine

According to design considerations and Solidworks drawings, a low-cost small scale de-oiling machine were developed (Fig 10). Specifications of the major parts of the fabricated vacuum frying machine are shown in Table 20. The developed machine is constructed with low cost parts

and easy to operate. The spare parts of the machine are available and affordable to exchange. The small size de-oiling machine is feasible to move from one place to another easily.

Table 21. Specification of the de-oiling machine

Sl. No.	Parameter	Dimension/Specification
01.	Base dimension	(560×350×360) mm
02.	Dimension of the de-oiling chamber	(240×230) mm
03.	Dimension of the oil drain channel	(250×30×20) mm
04.	Dimension of the lid	(100×20) mm
05.	Power of the motor	1.5 Hp
06.	Centrifugal speed	1400 rpm
07.	Capacity of the chips basket	600-800g/batch

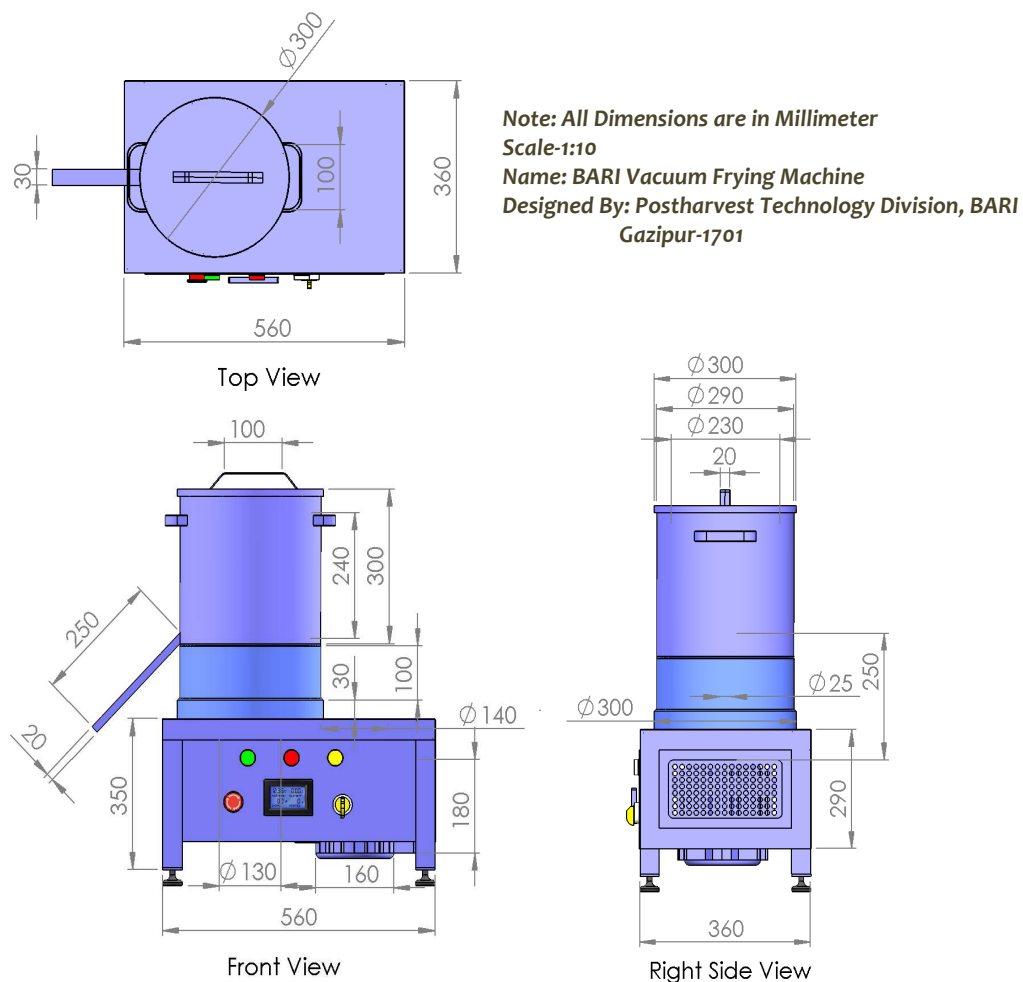


Fig 10. Schematic view of BARI developed de-oiling machine.

11.3.4.1. Working principle of the de-oiling machine

A simplified de-oiling machine was fabricated for releasing excessive oil from the fried chips product (specifications are shown in Table 21). When the basket rotates with high speed clockwise the external force is created and the products attached with the perforated wall of the basket and for pressure gradient the high density oil get rid of ridges and pores of the fried chips through capillary. Advanced centrifugation principle adopts to maximize de-oiling from the surface of the fried chips product. The oil reduction percentage is associated with the increased in rotational speed and time of the de-oiling machine due to comprehensive removal of surface oil through the pores (Plate 16).



Plate 16. Locally fabricated BARI de-oiling machine.

11.3.5. Formulation development for vacuum fried Banana chips

Consumer satisfaction depends on the quality of the product whereas the product appearance is the most common property to make positive or negative impression for the consumer. In this regard suitable spices combination was set by using different spices at different ratio (Table 22). Consumers' preference of spices combination presented in Table 23 & Plate 17.

Table 22. Spices combination of fried chips product

Name of ingredients	Ingredient of spices (%)				
	Type-A	Type-B	Type-C	Type-D	Type-E
Salt powder	Control treatment (without spices)	28.57	41.67	20.00	80.00
Sugar powder		47.62	47.67	40.00	-
Garlic powder		23.81	8.33	-	-
Chili powder		-	8.33	-	20.00
Capsicum powder		-	-	40.00	-

Table 23. Consumer preference test of mixed spices for vacuum fried banana chips

Spices combination	Sensory attributes				
	Appearance	Taste	Aroma	Crispiness	Overall acceptability
A	6.90	6.90	7.00	7.50	7.08
B	7.20	7.30	6.90	7.30	7.17
C	7.50	7.40	7.20	7.40	7.38
D	7.40	7.40	7.00	7.50	7.32
E	7.10	6.40	6.10	6.60	6.55

Hedonic scale: 9= Like extremely, 8= like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1=Dislike extremely

**Plate 17.** Sensory evaluation of suitable spices combination selection.

11.3.5.1. Evaluation of storage study for the vacuum fried banana chips product

Visual color of a product is a major quality criterion for determining the commercial quality with respect to consumers' preferences and marketability of the chips. Consumer perception of processed products depends mostly on the appearance and organoleptic characteristics. The products color may vary from process to process. To develop vacuum fried banana chips in the pretreatments, dipping solution of lemon juice and turmeric power was performed to exhibit natural color and to preserve the raw chips. In that case, white banana flesh turned into bright yellow color and after frying, it turned into deep yellow to bright yellow. It was observed that during vacuum frying, processed raw material contained high moisture content so frying time was increased to obtain the optimum moisture content in the product. It was noted in Table 24 with the increasing of frying temperature and time banana chips color became lighter due to increasing glossiness of the products. The lowest lightness (L^*) (65.96) was observed in treatment T_1 (110 °C & 8 min) because of incomplete frying and oil absorption of fried banana chips and the highest (76.43) was noticed in treatment T_9 (130 °C & 12 min). It was occurred due to longer frying time with higher temperature so de-pigmentation was happened. Lightness value indicated the significant differences with different frying temperature and time. It was also

stated that the lightness of fried chips became darker during product stored in HDPE packet where moisture was absorbed in the fried chips. The lightness values of all treatments became decreased from their initial value with storage (Table 24). The changes in color during frying were the results of starch gelatinization and non-enzymatic browning reactions (Richardson and Hyslop, 1985; Shyu and Hwang, 2001; Garayo and Moreira, 2002).

Table 24. Effect of frying temperature-time on lightness (L*) value of vacuum fried banana chips during 6 months of storage at ambient condition

Treatments	Lightness (L*) value			
	Initial	2 months	4 months	6 months
T ₁	65.96±0.76d	59.18±0.55c	54.46±1.14d	49.45±0.86c
T ₂	70.18±1.42cd	61.46±0.35bc	56.09±1.43cd	52.02±0.38bc
T ₃	72.92±0.08bc	64.66±1.09b	62.45±0.82bc	64.88±0.53a
T ₄	71.28±0.94bc	71.57±1.11a	68.49±0.78ab	55.11±0.93b
T ₅	72.68±0.77bc	71.65±0.98a	67.57±0.61ab	65.02±0.35a
T ₆	75.91±1.82a	72.23±1.02a	70.27±0.75a	64.88±0.64a
T ₇	72.27±1.57abc	72.73±0.58a	69.52±0.26ab	63.87±1.62a
T ₈	75.29±1.20ab	72.78±1.40a	72.86±0.81a	65.29±0.11a
T ₉	76.43±0.85a	73.90±0.30a	69.52±0.98ab	69.44±1.17a

Note: T₁= Temperature 110° C & time 8 min, T₂= Temperature 110° C & time 10 min, T₃= Temperature 110° C & time 12 min, T₄= Temperature 120° C & time 8 min, T₅= Temperature 120° C & time 10 min, T₆=Temperature 120° C & time 12 min, T₇= Temperature 130° C & time 8 min, T₈= Temperature 130° C & time 10 min, T₉= Temperature 130° C & time 12 min.
All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d indicates significant result (p<0.05).

Chroma is the departure degree of a color from the neutral color of the same value. Chroma, measured radially from the center of each slice, represents the “purity” of a color (related to saturation), with lower chroma being less pure. Therefore, the lowering of the chroma value indicated the lowering of the bright color into blemishes. There were significant changes in the chroma value for each treatment along with storage period also. In Table 25, treatment T₁ showed the more deviation from the initial chroma value 41.58 to 29.39 whereas treatment T₆ retained the more natural color after 6 months of storage.

This variation might be due to the differences in the transmittance of light through the Nano pores of the packages used in the experiment. Generally, PP films are clear and more transparent than other polymer films. Hence, light is able to enter into the package and influence the color of the packaged material (Kirwan and Strawbridge, 2003).

Table 25. Effect of frying temperature-time on chroma (C*) value of vacuum fried banana chips during 6 months of storage at ambient condition

Treatments	Chroma (C*) value			
	Initial	2 months	4 months	6 months
T ₁	41.58±0.11c	30.86±1.20b	33.35±0.07c	29.39±0.35b
T ₂	42.98±1.31bc	41.22±1.14a	41.99±0.15a	34.00±0.62ab
T ₃	43.72±1.01abc	35.31±0.84b	36.62±0.20bc	33.45±0.15ab
T ₄	45.20±0.46abc	43.35±0.59a	40.83±1.23ab	33.46±0.88ab
T ₅	45.11±0.76abc	45.59±0.70a	42.76±0.25a	35.32±0.50ab
T ₆	45.71±0.72ab	44.49±0.46a	43.33±0.65a	38.57±0.25a
T ₇	45.20±0.03abc	43.29±1.09a	40.70±0.55ab	33.27±1.01ab
T ₈	46.98±1.21ab	41.43±1.24a	40.92±1.38ab	34.00±0.57ab
T ₉	47.06±0.20a	43.23±0.9a	41.12±0.59ab	33.58±1.36ab

Note: T₁= Temperature 110° C & time 8 min, T₂= Temperature 110° C & time 10 min, T₃= Temperature 110° C & time 12 min, T₄= Temperature 120° C & time 8 min, T₅= Temperature 120° C & time 10 min, T₆= Temperature 120° C & time 12 min, T₇= Temperature 130° C & time 8 min, T₈= Temperature 130° C & time 10 min, T₉= Temperature 130° C & time 12 min. All values are means of triplicate determinations ±SD. Means within columns with different letters a, b, c indicate significant result (p<0.05).

Table 26. Effect of frying temperature-time on moisture content (%) of vacuum fried banana chips during 6 months of storage at ambient condition

Treatments	Moisture content (%)			
	Initial	2 months	4 months	6 months
T ₁	8.69±0.05a	8.87±0.07a	9.87±0.05a	9.88±0.01a
T ₂	5.40±0.02b	5.55±0.05b	5.83±0.02b	5.91±0.06b
T ₃	3.96±0.03c	4.27±0.02c	4.33±0.03c	4.98±0.03c
T ₄	4.06±0.05c	4.18±0.01c	4.60±0.02d	5.26±0.06b
T ₅	2.54±0.08d	2.74±0.02d	3.55±0.01e	4.62±0.03e
T ₆	2.31±0.1d	2.65±0.01d	3.46±0.02f	4.03±0.01f
T ₇	2.49±0.18d	2.67±0.06d	3.27±0.05g	4.60±0.01e
T ₈	1.24±0.07e	1.86±0.07e	3.27±0.01g	4.56±0.01e
T ₉	1.20±0.03e	1.62±0.01f	3.25±0.02g	4.08±0.02f

Note: T₁= Temperature 110° C & time 8 min, T₂= Temperature 110° C & time 10 min, T₃= Temperature 110° C & time 12 min, T₄= Temperature 120° C & time 8 min, T₅= Temperature 120° C & time 10 min, T₆= Temperature 120° C & time 12 min, T₇= Temperature 130° C & time 8 min, T₈= Temperature 130° C & time 10 min, T₉= Temperature 130° C & time 12 min. All values are means of triplicate determinations ±SD. Means within columns with different letters a, b, c, d, e, f, g indicate significant result (p<0.05).

Initially the moisture content of fresh banana was observed 86%. From the observation, it was found that an increase in the oil temperature resultant in a significant reduction (p<0.05) of moisture retention of the fried banana chips with the same pressure. In Table 26, it was noticed that the chips fried at lowest temperature in treatment T₁ (110 °C for 8 min) contained the highest moisture content (8.69%) than chips fried at highest temperature in treatment T₉ (130 °C for 8 min) (1.20%). During storage, as the fried banana chips were packaged in HDPE packet there was the absorption of moisture by penetration where the moisture content attained prominently from 4.02 to 9.88% among the treatments.

In case of firmness, the maximum was observed in treatment T_1 maximum (0.54 N) and the minimum was found in treatment T_3 (0.26 N). The increase in breaking force at the end of storage period was 0.93 N and 1.28 N in the treatments T_9 and T_1 respectively (Table 27). The increase in moisture content and water activity during the storage period might have influenced the breaking force. Hence, the breaking force is directly influenced by water vapor transmission characteristics of film. Ammawath *et al.*, (2002) observed the increase in breaking force of banana chips, which was stored in polypropylene film packet during storage.

Table 27. Effect of frying temperature-time on firmness (N) of vacuum fried banana chips during 6 months of storage at ambient condition

Treatments	Firmness (N)			
	Initial	2 months	4 months	6 months
T_1	0.54±0.03a	1.14±0.09a	1.16±0.12a	1.28±0.06a
T_2	0.28±0.03cd	0.64±0.02bc	1.10±0.11a	1.25±0.20a
T_3	0.26±0.04d	0.51±0.06cd	0.58±0.13c	0.84±0.01b
T_4	0.38±0.03b	0.59±0.04cd	0.70±0.03bc	0.95±0.03b
T_5	0.35±0.04bc	0.60±0.03bcd	0.70±0.03bc	0.82±0.03b
T_6	0.32±0.03bcd	0.48±0.05d	0.73±0.01bc	0.92±0.04b
T_7	0.33±0.02bc	0.75±0.05b	0.95±0.11b	0.98±0.05b
T_8	0.32±0.02bcd	0.52±0.03cd	0.76±0.09bc	0.95±0.01b
T_9	0.31±0.01bcd	0.62±0.04bcd	0.80±0.06bc	0.93±0.01b

Note: T_1 = Temperature 110° C & time 8 min, T_2 = Temperature 110° C & time 10 min, T_3 = Temperature 110° C & time 12 min, T_4 = Temperature 120° C & time 8 min, T_5 = Temperature 120° C & time 10 min, T_6 = Temperature 120° C & time 12 min, T_7 = Temperature 130° C & time 8 min, T_8 = Temperature 130° C & time 10 min, T_9 = Temperature 130° C & time 12 min. All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d indicate significant result ($p < 0.05$).

Vitamin C (Ascorbic acid) is an important nutritional parameter for fried food products. Thermal degradation of total ascorbic acid increased with increasing frying temperature-time of fried banana chips. Reduction in ascorbic acid content is possible in the absence of oxygen and at relatively low frying temperatures as it can follow an anaerobic pathway of non-enzymatic browning reactions (Dueik and Bouchon, 2011). It was observed the similar results that vitamin C reduced significantly in all treatments in banana chips during storage period. The highest ascorbic acid was obtained in treatment T_1 (15.23 mg/100 g) and the lowest was found in treatment T_9 (12.29 mg/100 g). During 6 months of storage, the ascorbic acid content reduced drastically for all treatments (Table 28). It might be due to influence of light, oxidation and metabolic action. The loss of vitamin C loss was described a linear relationship with temperature in vacuum-fried gold kiwi fruit (Diamante *et al.*, 2013).

Table 28. Effect of frying temperature-time on vitamin C content of vacuum fried banana chips during 6 months of storage at ambient condition

Treatments	Vitamin C			
	Initial	2 months	4 months	6 months
T ₁	15.23±0.55bc	8.03±0.50d	5.52±0.27d	2.27±0.25e
T ₂	19.22±0.99a	14.67±0.64a	5.88±0.01d	2.02±0.12e
T ₃	15.85±0.30b	15.22±1.42a	5.81±0.07d	3.46±0.44cd
T ₄	15.21±0.50bc	12.09±0.32b	5.85±0.03d	3.39±0.18cd
T ₅	13.75±0.49cd	12.25±0.05b	9.17±0.73b	4.38±0.44ab
T ₆	14.97±0.50bc	12.87±0.32b	10.10±0.18a	4.83±0.08a
T ₇	13.20±0.78d	11.30±0.14bc	9.09±0.52b	4.95±0.22a
T ₈	12.59±0.25d	8.17±0.31d	5.82±0.05d	4.02±0.21bc
T ₉	12.29±0.21d	9.57±0.56cd	6.90±0.12c	3.25±0.12d

Note: T₁= Temperature 110° C & time 8 min, T₂= Temperature 110° C & time 10 min, T₃= Temperature 110° C & time 12 min, T₄= Temperature 120° C & time 8 min, T₅= Temperature 120° C & time 10 min, T₆= Temperature 120° C & time 12 min, T₇= Temperature 130° C & time 8 min, T₈= Temperature 130° C & time 10 min, T₉= Temperature 130° C & time 12 min. All values are means of triplicate determinations ±SD. Means within columns with different letters a, b, c, d indicate significant result ($p<0.05$).

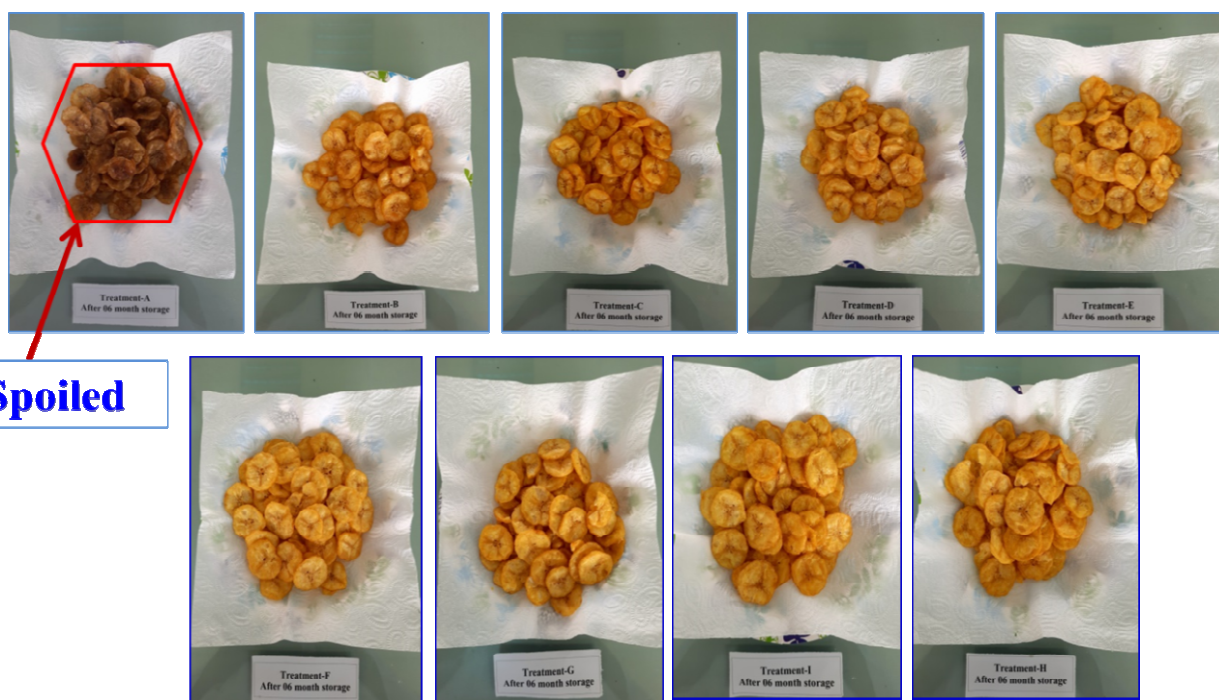
**Plate 18.** Vacuum fried banana chips stored in HDPE packet at ambient temperature after 6 months of storage.



Plate 19. Sensory evaluation of the vacuum fried banana chips.

Table 29. Consumer preference test of the vacuum fried banana chips

Treatment	Sensory attributes									
	Initial					After 6 months				
	AP R	Taste	Aroma	CRS	OAT	APR	Taste	Aroma	CRS	OAT
T ₁	6.0	3.6	5.0	2.8	4.4	Spoil	Spoil	Spoil	Spoil	spoil
T ₂	7.0	5.9	6.6	5.4	6.2	6.8	5.0	5.1	4.3	5.3
T ₃	7.4	7.0	7.6	7.2	7.3	7.0	5.4	6.3	4.9	5.9
T ₄	7.4	7.0	7.6	7.0	7.3	6.8	6.4	5.9	6.0	6.3
T ₅	7.2	7.0	7.6	7.0	7.2	7.2	6.5	6.0	6.1	6.5
T ₆	8.0	7.8	8.2	8.6	8.2	7.3	6.9	6.8	6.3	6.8
T ₇	7.6	7.8	7.8	8.2	7.9	7.2	6.3	6.4	6.3	6.6
T ₈	7.8	8.2	8.0	8.4	8.1	7.2	6.6	6.8	6.4	6.8
T ₉	7.8	8.2	7.6	8.6	8.1	7.0	6.8	6.8	6.5	6.8

Note: APR=Appearance, CRS=Crispiness, OAT=Overall acceptability, T₁= Temperature 110° C & time 8 min, T₂= Temperature 110° C & time 10 min, T₃= Temperature 110° C & time 12 min, T₄= Temperature 120° C & time 8 min, T₅= Temperature 120° C & time 10 min, T₆= Temperature 120° C & time 12 min, T₇= Temperature 130° C & time 8 min, T₈= Temperature 130° C & time 10 min, T₉= Temperature 130° C & time 12 min. Hedonic scale: 9= Like extremely, 8= like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1=Dislike extremely.

Hedonic scale: 9= Like extremely, 8= like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1=Dislike extremely.

In Table 29, it was observed that at initial stage the highest overall acceptability score was observed in treatments T₆ (120°C & 12 min) (8.2) and the lowest was found in treatment T₁ (110°C & 8 min) (4.4). After 6 months of storage, the maximum overall acceptability (6.8) was noticed in treatment T₆ (120°C & 12 min), T₈ (130°C & 10 min), T₉ (130°C & 12 min) by the expert panelists. After 6 months storage, treatment T₁ (110°C & 8 min) was found spoiled due to higher moisture content, less crispiness and fungal growth in the products.

11.3.6. Result and discussion of vacuum fried sweet potato chips product

For developing vacuum fried sweet potato chips four pre-treatments were set (Fig 12 & 13) as preliminary study to find out the optimum processing parameter. Different frying time and temperature also were observed. Sensory evaluation was conducted to determine the best

treatment. Without blanching and no sugar soaking performed most suitable treatment because of good appearance, texture and crispiness properties. Among four treatments, treatment B ranked the highest score 7.44 as shown in Table 30 and then the experimentation for shelf life study was conducted based on that treatment.

Table 30. Consumer preference test of pre-treated vacuum fried sweet potato chips

Treatment	Sensory attributes				
	Appearance	Taste	Aroma	Crispiness	Overall acceptability
A	7.10	6.98	7.00	7.36	7.11
B	8.00	7.30	7.10	7.56	7.44
C	5.60	6.10	5.96	6.80	6.12
D	6.20	6.56	6.50	6.90	6.54

Hedonic scale: 9= Like extremely, 8= like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1=Dislike extremely

The frozen sweet potato slices were fried in vacuum fryer from 2 mins to 14 mins at 2 mins interval at different frying temperature to obtain the equilibrium moisture. In Figure 30, it was observed that the moisture loss during vacuum frying exhibited a classical drying profile. As frying time increased, the moisture content rapidly decreased during frying. Among the frying temperature-time combination, the optimum frying time was found at 12 mins for all frying temperatures.

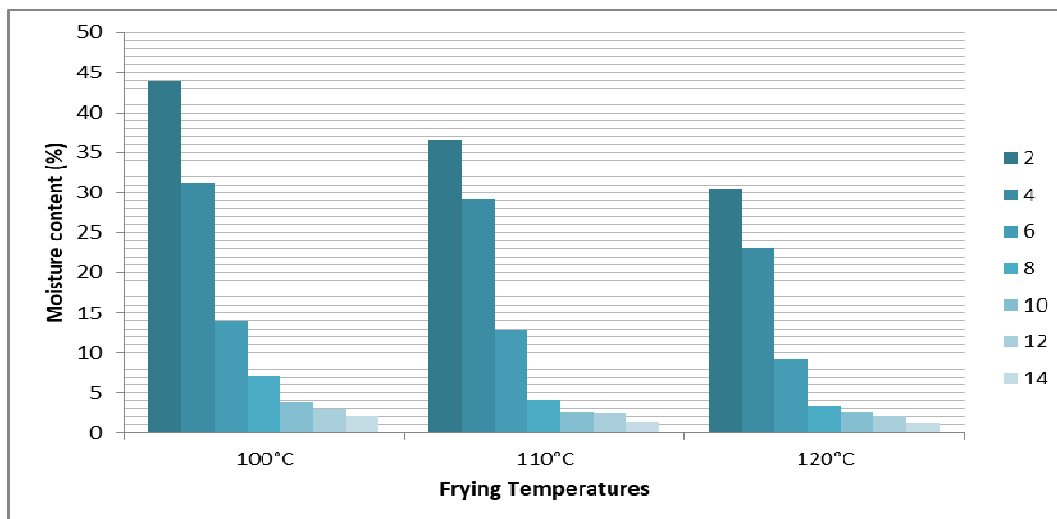


Fig 12. Changes of moisture (%) of vacuum fried sweet potato chips fried at different frying temperature-time combination.

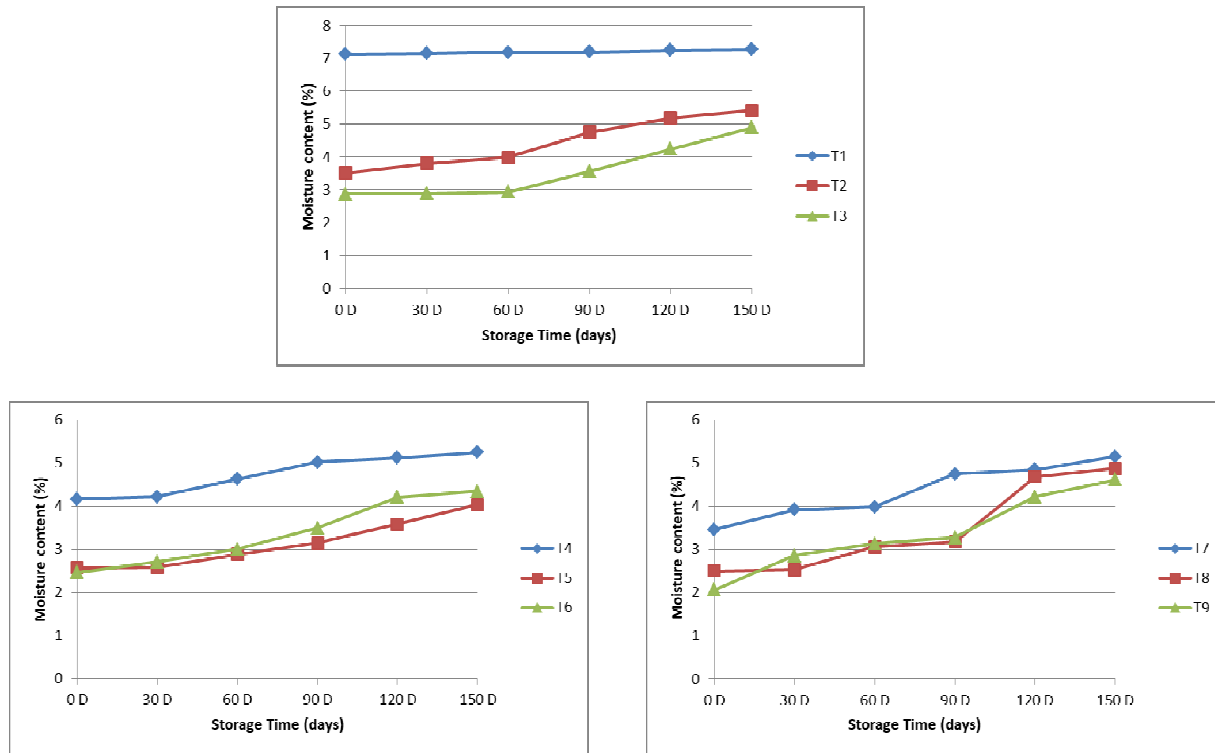


Fig 13. Changes of moisture (%) of vacuum fried sweet potato chips at different frying temperature-time combination during 5 months storage.

The moisture loss from sweet potato slices under vacuum at different oil temperatures (100, 110 and 120 °C) and time (8, 10 and 12 min) is shown in Fig 14. The phenomenon is in accordance with the findings for vacuum fried sweet potato chips it can be conclude that there were significant ($p < 0.05$) differences in moisture content. In Fig 13, it was observed that moisture content increased linearly during 5 months of storage period. It might be increased due to the penetration of environmental air to HDPE packet to turn into equilibrium condition. Moisture content increased from 2.06 % to 4.61 % in treatment T₉ and 7.12 % to 7.25 % in treatment T₁.

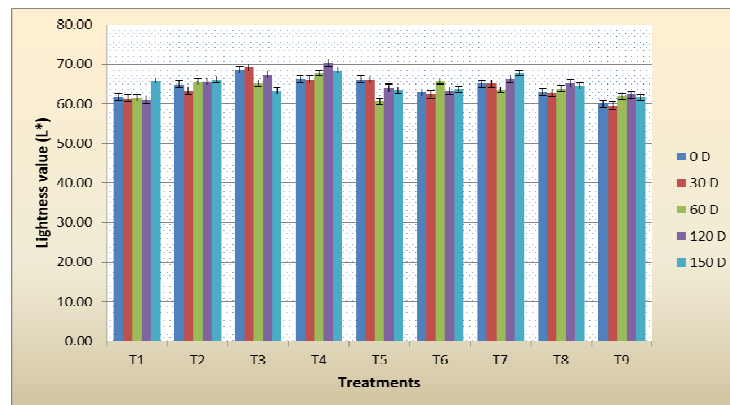


Fig 14. Effects of Lightness (L*) value of vacuum fried sweet potato chips at different temperature-time combination during 5 months of storage .

Sweet potato chips were fried at different frying time and temperatures that influences the lightness (L^*) of the vacuum fried chips products. The lightness of the chip's product changes with the changing of frying time and temperatures. At lower frying time and lower temperatures, the sweet potato chips color remains white or light, but with the increase of the time and temperature lightness was decreased in all treatments. Lightness value was insignificant difference among the treatments at each treatment. The lightness value ranges from 60.01-68.63 at initial day. The best color was observed at frying temperature 100 °C and time 12 minutes the products are more light color (68.63 ± 0.53) lower at temperature 120 °C for 12 minutes the lightness lower (60.01 ± 0.30) and day after storage of 5 months lightness turned into lowered gradually (Fig 15).

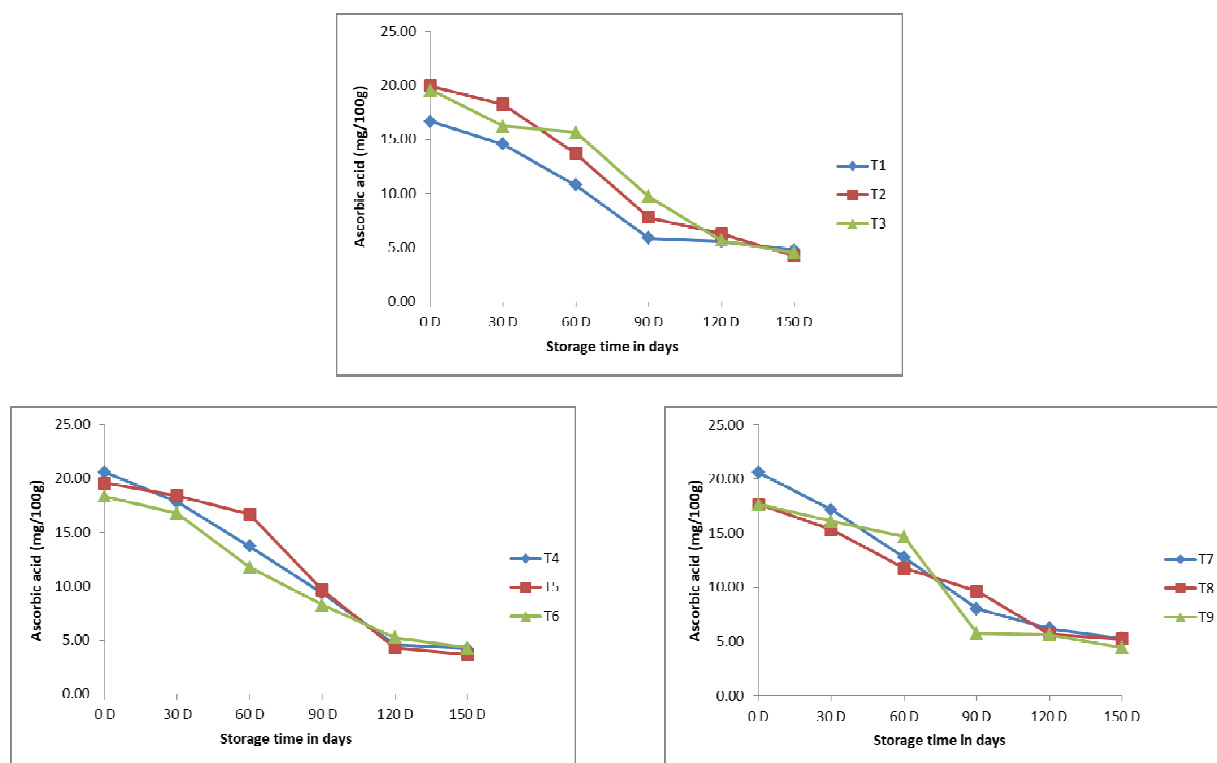


Fig 15. Ascorbic acid content (mg/100g) changes with different frying temperature-time combination during 5 months of storage.

Ascorbic is highly sensitive to heat and storage condition. In the Fig 15, it can be concluding that the ascorbic acid content reduced drastically with different frying temperature-time and storage period. The higher amount of ascorbic acid was observed in low temperature and low frying time at 100 °C, 10 min (22.53 ± 1.19 mg/100g) and lower ascorbic acid content at high temperature and higher time at 120 °C and 12 min (17.64 ± 0.00 mg/100g). After storage of 150 days the ascorbic acid content reduces significantly and the lowest was noticed in each treatment. Thermal degradation is a major cause of ascorbic acid content reduction. Reduction in ascorbic acid content is possible in the absence of oxygen and at relatively low frying temperatures as it could follow an anaerobic pathway of non-enzymatic browning reactions (Dueik and Bouchon, 2011).

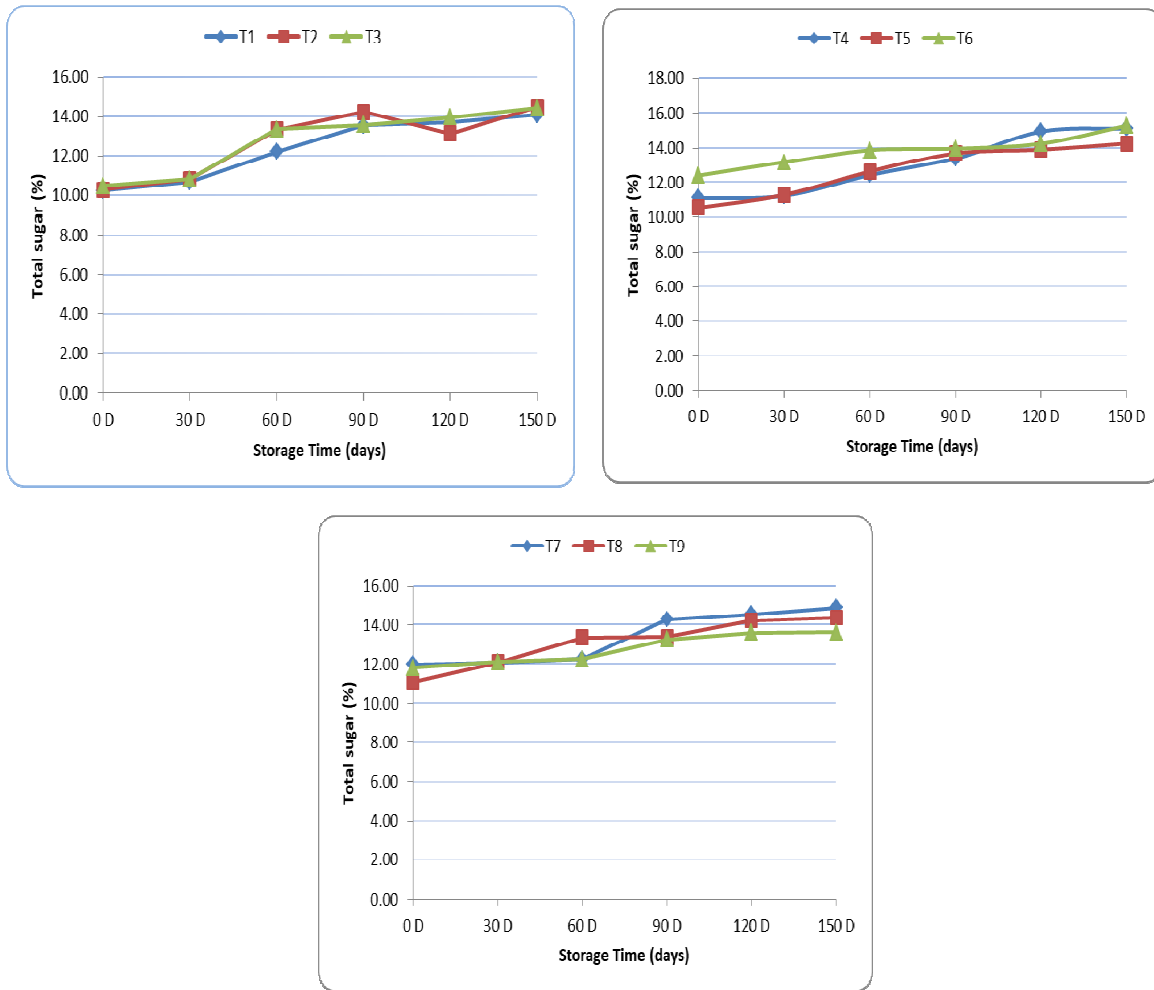


Fig 16. Total sugar content (%) changes with different frying temperature-time combination during 5 months of storage.

The changes in the total sugar content in sweet potato chips figure out in line diagram in Fig 16. From the study it was observed that at initial stage the total sugar content has no significant differences. But during storage it started to increase gradually in each treatment which was ranges from 10.24% to 14.10% in treatment T₁ and 12.39% to 15.30% in treatment T₆. During storage the starch starts to breakdown and converted into sugar due to physiological changes that's why the total sugar content increases.

In case of consumer preference test the highest overall acceptability, score was observed by expert panelist in treatment T₆ (6.90) (110 °C & 12 min) and the lowest was found in treatment T₁ (4.90) (100° C & 8 min). The sensory score (Table 31) indicated that after 5 months of storage treatment T₆ retained more sensory quality among the treatments at ambient temperature (Plate 20 & 21).



Plate 20. Sensory evaluation of vacuum fried sweet potato chips during 5 months of storage.

Table 31. Consumer preference test of vacuum fried sweet potato chips after 5 months of storage

Treatment	Sensory attributes				
	Appearance	Taste	Aroma	Crispiness	Overall acceptability
T ₁	5.40	4.90	4.60	4.70	4.90
T ₂	6.60	5.60	5.90	5.60	5.93
T ₃	7.40	7.10	6.50	6.20	6.80
T ₄	6.20	5.40	5.50	5.40	5.63
T ₅	6.40	6.10	5.80	5.80	6.03
T ₆	7.10	6.50	6.90	7.10	6.90
T ₇	6.30	5.60	5.00	5.00	5.48
T ₈	6.70	6.10	6.00	5.60	6.10
T ₉	6.90	6.70	5.70	6.40	6.43

Note: T₁= Temperature 100° C & time 8 min, T₂= Temperature 100° C & time 10 min, T₃= Temperature 100° C & time 12 min, T₄= Temperature 110° C & time 8 min, T₅= Temperature 110° C & time 10 min, T₆= Temperature 110° C & time 12 min, T₇= Temperature 120° C & time 8 min, T₈= Temperature 120° C & time 10 min, T₉= Temperature 120° C & time 12 min.

Hedonic scale: 9= Like extremely, 8= like very much, 7= Like moderately, 6= Like slightly, 5= Neither like or dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1=Dislike extremely

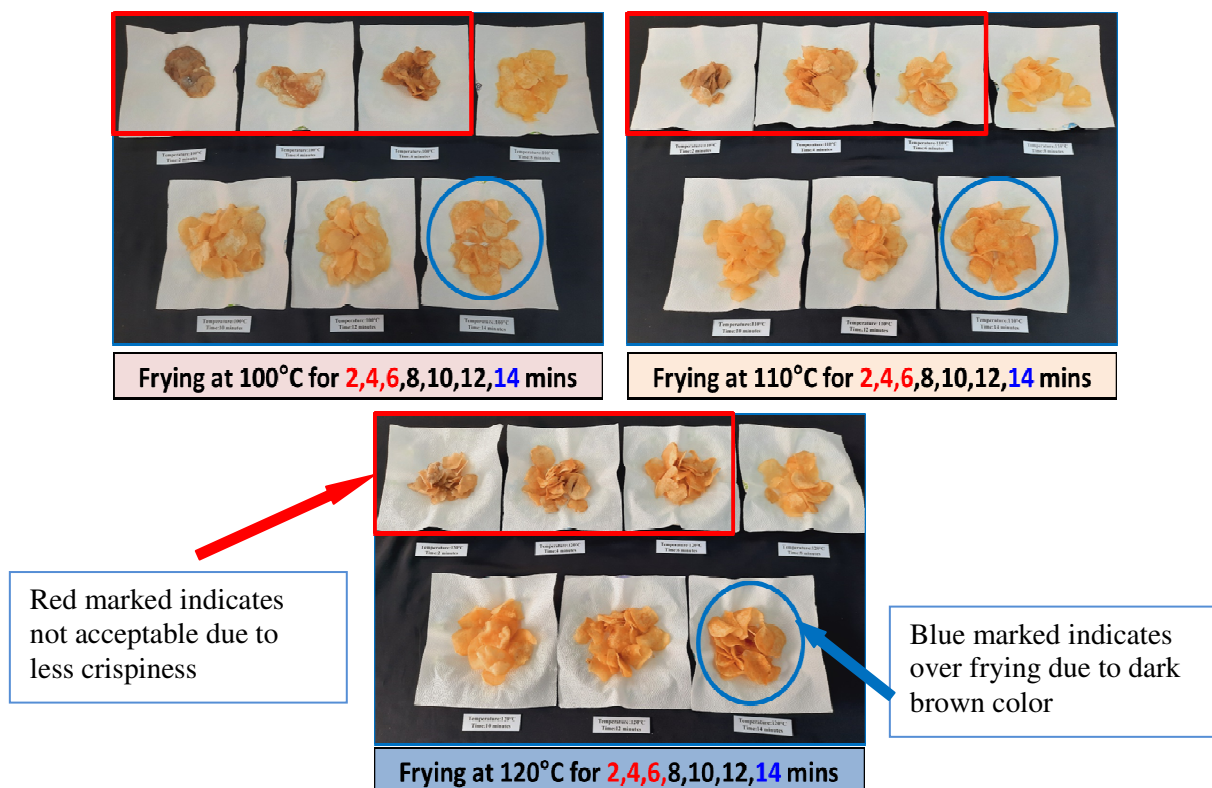


Plate 21. Vacuum fried Sweet potato chips stored in HDPE packet at ambient temperature after 5 months of storage.

11.3.7. Results and discussion of the vacuum fried Jackfruit chips product

In this experiment, standardization of pre-treatments was done to produce good quality vacuum fried jackfruit chips. Freezing at -18°C was carried out as pretreatment and three different frying temperatures (100°C, 110°C and 120°C) at 5 minutes frying interval (0 to 25 min) used for optimization and development of vacuum frying of jackfruit chips. Each sample was stored at ambient temperature in Metalex foil pack.

The color values of vacuum fried (VF) jackfruit chips showed significant difference with various frying temperature and time combination. The Lightness (L^*) values of VF jackfruit chips are shown in Table 32. The L^* values of the VF jackfruit chips ranged from 54.17 to 67.48 when fried at different frying temperature and time. The L^* values were seen to be inversely proportional to the frying temperature. A higher L^* value was observed in VF jackfruit chips at frying condition of 100°C for 20 min. Lower L^* value of 54.17 was observed in the VF jackfruit chips at processing conditions of 100°C for 5 min. When frying time was further extended, the L^* value decreased at all frying temperatures. Frying at 120°C for 25 minutes the lightness decreases to 57.89. During storage the lightness values decreases 44.17 to 62.53 and the products became darker in color. The change in color was due to the interaction of an amine group with a reducing sugar, which is a non-oxidative browning reaction, pigment fragmentation and oxidation (Mariscal and Bouchon, 2008).

Table 32. Effect of frying temperature-time on lightness (L^*) value of VF Jackfruit chips during 6 months of storage at ambient condition

Treatment		Lightness (L^*) value			
Temperature	Time (min)	Initial	2 months	4 months	6 months
100°C	5	59.17±0.41f	47.69±0.49f	46.41±0.50f	44.77±0.47d
	10	62.63±0.65bc	55.23±0.94de	48.39±0.65f	45.23±0.03d
	15	64.98±0.26ab	63.19±0.34ab	57.81±0.41bcd	54.36±0.15bc
	20	67.48±0.95a	62.29±0.68ab	57.98±0.66bcd	54.07±0.13bc
	25	62.44±0.73ab	62.46±0.06ab	60.14±0.77abc	58.53±0.49ab
110°C	5	56.76±0.51ef	52.34±0.42e	47.52±0.27f	44.14±1.57d
	10	62.52±0.57bc	52.26±0.82e	46.46±0.39f	48.27±1.17cd
	15	63.96±0.32ab	61.19±0.68abc	56.83±0.54cd	55.55±0.56b
	20	63.34±0.06ab	60.49±0.48bc	58.11±0.20bcd	55.89±0.60b
	25	62.95±0.40bc	62.35±1.01ab	61.46±0.84ab	60.05±0.05ab
120°C	5	60.37±0.87cd	57.96±0.21cd	52.56±0.62e	45.86±0.08d
	10	65.05±0.34ab	61.84±1.06ab	57.64±0.92cd	55.18±0.03b
	15	66.23±0.50a	63.97±1.73a	62.40±0.10a	62.53±0.29a
	20	65.38±0.06ab	63.22±0.93ab	63.81±0.48a	59.56±0.40ab
	25	57.89±0.09de	56.62±0.54d	55.74±0.64de	54.85±0.15bc

All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f indicates significant result ($p < 0.05$).

The a^* value of the vacuum fried chips was found to increase with the progress of frying duration at all the frying temperatures (Table 33). The a^* value ranges from 2.16 to 4.94. The increase was very rapid at 120°C compared to other frying temperatures. During storage a^* values starts to increase in all treatments. After six months storage a^* value ranges from 5.96 to 8.77. The major changes observed in low temperatures 100°C and 110°C and low frying time 5 and 10 minutes respectively. Changes in a^* value indicated development of golden brown to dark brown color in jackfruit chips due to incomplete frying with higher moisture level that causes browning reactions, breakdown of the chemical compounds and fungal growth (Garayo and Moreira, 2002).

Table 33. Effect of frying temperature-time on a^* value of VF Jackfruit chips during 6 months of storage at ambient condition

Treatment		a^* value [(+ve) redness and (–ve) greenness]			
Temperature	Time (min)	Initial	2 months	4 months	6 months
100°C	5	2.16±0.02g	5.14±0.08bc	7.41±0.11a	8.77±0.15a
	10	3.57±0.12ef	5.13±0.03bc	7.50±0.41a	8.64±0.13a
	15	3.61±0.14ef	4.05±0.16g	5.59±0.46ef	5.95±0.03f
	20	4.38±0.24cd	4.85±0.04cd	5.58±0.05ef	5.96±0.02f
	25	4.76±0.29ab	5.08±0.04bc	5.66±0.02ef	5.85±0.22f
110°C	5	3.43±0.41f	5.55±0.23a	7.21±0.03a	8.21±0.12a
	10	3.84±0.04ef	4.36±0.19efg	7.15±0.21a	8.84±0.05a
	15	3.91±0.05ef	4.27±0.06fg	6.17±0.04cde	7.02±0.02b
	20	4.52±0.11abc	4.94±0.02bcd	6.24±0.03cd	6.47±0.07bc
	25	4.90±0.06a	5.07±0.02bc	5.64±0.13def	6.28±0.13de

120°C	5	3.46±0.01f	4.51±0.07ef	6.16±0.14cde	7.74±0.17a
	10	3.54±0.11ef	5.19±0.08b	6.53±0.09bc	7.05±0.04a
	15	4.03±0.03cde	5.10±0.09bc	6.12±0.08cd	6.66±0.36bcd
	20	4.37±0.09bcd	4.66±0.03de	5.17±0.36f	6.60±0.13cd
	25	4.94±0.05a	5.12±0.02bc	6.33±0.05c	6.73±0.04bc

All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f, g indicates significant result (p<0.05).

The moisture loss from the jackfruit bulbs under vacuum at different frying temperature and time is illustrated in Table 34. There were significant ($P<0.05$) differences in moisture content in the vacuum fried Jackfruit chips. From the observation, the result indicates that at each temperature frying at 5 and 10 minutes produces incomplete fried chips due to less crispiness. The moisture content of the jackfruit chips were ranges from 40.38% to 1.83% at 100°C for 5 minutes and 120°C for 25 minutes respectively. Since the frying is carried out under vacuum which decreases the boiling point of water, so moisture removal was instant without much warm-up. After storage the moisture content increased in each treatment ranges 3.25% to 44.11% .due to the absorbance of moisture with microspores of the packaging materials.

Table 34. Effect of frying temperature-time on moisture content (%) of VF Jackfruit chips during 6 months of storage at ambient condition

Treatment		Moisture (%)			
Temperature	Time (min)	Initial	2 months	4 months	6 months
100°C	5	40.83±1.91a	41.18±0.18a	42.17±0.15a	44.11±0.10a
	10	8.11±0.59d	10.24±0.31d	10.60±0.24c	11.81±0.13d
	15	3.61±0.23ef	4.34±0.18f	5.02±0.02e	5.96±0.06f
	20	2.69±0.03ef	2.84±0.02g	3.28±0.14f	3.73±0.06h
	25	2.60±0.08ef	2.87±0.01g	3.19±0.00fg	3.70±0.01h
110°C	5	27.18±0.31b	27.15±0.94b	29.46±0.18b	29.78±0.18b
	10	6.39±0.37d	8.55±0.32e	8.62±0.05c	8.95±0.05e
	15	3.09±0.09ef	4.59±0.13f	5.84±0.04d	5.96±0.03f
	20	2.73±0.10ef	2.92±0.03g	3.29±0.09f	3.71±0.02h
	25	1.88±0.06f	2.42±0.02g	2.92±0.03g	3.25±0.05i
120°C	5	18.88±1.13c	21.51±0.43c	22.34±0.14b	22.71±0.16c
	10	4.19±0.09e	4.70±0.25f	5.64±0.12d	6.04±0.28f
	15	3.08±0.13ef	4.20±0.02f	4.93±0.04e	5.32±0.02g
	20	2.29±0.16f	2.64±0.04g	3.08±0.04fg	3.42±0.14i
	25	1.83±0.04f	2.32±0.05g	2.88±0.01g	3.41±0.07i

All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f, g, h, i indicate significant result (p<0.05).

There were significant differences observed in starch content in the jackfruit chips fried at different temperature and time. During frying starch becomes gelatinized due to heating and the products becomes crisps in nature. The starch content ranges from 15.91% to 17.88% after frying. Jackfruit bulbs fried at lower temperature for lower time causes higher starch content 17.88% due to incomplete frying but it decays drastically up to 13.24 %during storage of six months. During storage starch starts breaks down ranges from 10.15% to 13.25% by converting into sugar. The starch content in the jackfruit chips is illustrated as bar diagram in Fig 17.

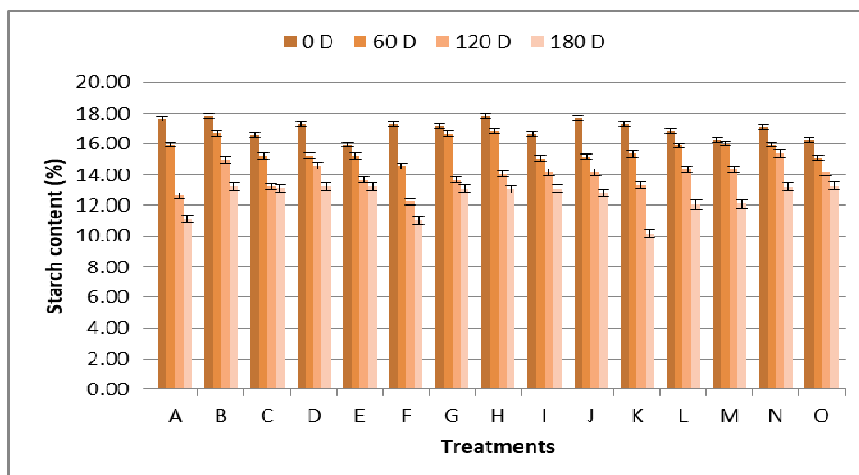


Fig 17. Changes of starch (%) of VF Jackfruit chips during 6 months storage at ambient temperature.



Plate 23. Keeping quality of VF Jackfruit chips storage after 6 months.

Vacuum fried jackfruit chips were assessed for sensory acceptability in terms of appearance, taste, flavor, crispiness, oiliness and overall acceptability (Table 35). The sensory score for jackfruit color was rated high during chips frying at higher temperature with time. It was observed that yellow flesh turned into golden yellow during vacuum frying. Jackfruit chips fried at higher temperature 120°C with longer frying time (over 25 min) resultant lowering the sensory score due to over frying and undesirable surface browning of the slices, which occurred caramelization of sugar. Higher frying temperature exhibited crispiness faster. In case of sensory evaluation, the highest overall acceptability was found in treatment J (6.56) and treatment N (6.15) respectively. In the experimentation, treatment A,B, C, F, G, H, K, L and M was not belongs to sensory score under 5 (Neither like or dislike) for the development of the VF jackfruit chips due to incomplete frying and less crispiness that resulted in the sensory score.

It can be concluded that suitable frying temperature-time combination is an important issue for quality vacuum fried chips products considering organoleptic properties (Table 35). Without suitable packaging materials, products quality attributes mainly texture and appearance were greatly affected. It was also observed that the shelf life of jackfruit chips was 2-3 months in HDPE packet and 6-8 months in nitrogen flashed foil packet. Without nitrogen products becomes quite rancid flavor due to oxidation of fat.

Table 35. Consumer preference test of vacuum fried jackfruit chips after 6 months of storage in HDPE packet

Treatment	Sensory attributes											
	Initial						After 6 months					
	APR	TAS	FLR	CRP	OLS	OAT	APR	TAS	FLR	CRP	OLS	OAT
A	3.60	2.80	3.00	1.40	1.40	2.44	1.00	1.00	1.00	1.00	1.00	1.00
B	6.40	5.40	5.00	4.00	4.50	5.06	1.20	1.00	1.20	1.40	1.60	1.28
C	7.10	6.70	6.60	7.20	7.60	7.04	4.40	3.50	3.40	5.60	5.60	4.50
D	7.90	7.80	7.50	8.10	8.10	7.88	5.20	4.20	4.00	6.30	6.20	5.18
E	7.80	7.10	7.80	8.00	8.00	7.74	5.60	5.50	5.20	7.04	6.90	6.05
F	5.80	3.60	3.20	2.20	2.80	3.52	1.20	1.00	1.20	1.00	1.00	1.08
G	7.20	7.70	7.30	4.80	6.44	6.69	1.00	1.00	1.20	1.00	1.00	1.04
H	6.80	5.80	6.40	5.40	7.10	6.30	4.70	4.00	3.50	5.40	6.00	4.72
I	8.10	7.40	7.80	8.24	8.10	7.93	4.80	5.00	4.40	6.74	7.00	5.59
J	7.30	8.20	8.00	8.20	8.10	7.96	6.20	6.00	5.70	7.50	7.40	6.56
K	6.40	3.20	4.40	2.20	4.60	4.16	1.40	1.00	1.00	1.00	1.00	1.08
L	7.80	7.00	7.44	7.20	7.44	7.38	3.80	3.40	3.20	4.80	5.60	4.16
M	7.50	7.20	7.30	7.50	7.80	7.46	4.80	4.00	3.80	5.90	6.20	4.94
N	7.70	7.80	7.80	8.20	7.90	7.88	5.80	5.30	5.00	7.14	7.50	6.15
O	6.90	7.44	7.04	8.20	7.90	7.50	4.60	4.50	4.10	6.50	6.70	5.28

Note: APR=Appearance, TAS=Taste, FLR=Flavor, CRP=Crispiness, OLS=Oiliness, OAT=Overall acceptability, A=Temperature 100° C & time 5 min, B=Temperature 100° C & time 10 min, C=Temperature 100° C & time 15 min, D=Temperature 100° C & time 20 min, E=Temperature 100° C & time 25 min, F=Temperature 110° C & time 5 min, G=Temperature 110° C & time 10 min, H=Temperature 110° C & time 15 min, I=Temperature 110° C & time 20 min, J=Temperature 110° C & time 25 min, K=Temperature 120° C & time 5 min., L=Temperature 120° C & time 10 min, M=Temperature 120° C & time 15 min, N=Temperature 120° C & time 20 min, O=Temperature 120° C & time 25 min.

Hedonic Scale: 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like or dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely.

11.4. Phytochemicals profiles of selected forage herbs

Considering the quantitative polyphenolic profile, and biomass yield, the following herbs were found to have a greater potential as feed additives for farm livestock: plantain (*Plantago lanceolata* L.), ivy gourd (*Coccinia grandis* L.), garlic (*Allium sativum*), mint (*Mentha* spp), and lemongrass (*Cymbopogon citratus*). According to TLC analysis, lemongrass contain all the selected polyphenolic components, whereas plantain and spear mint are deficient in saponin and glycoside, respectively (Table 36). Additionally, the level of specific bioactive components in selected herbs along with the variation due to different preservation methods were undertaken (Table 37), which will aid in determining the dose related response and sustainability of using these herbs in farm animals.

Table 36. Result of TLC analysis/Phytochemical screening of the medicinal herbs

Parameter	PL	GL	LG	IG	SM
Terpenoids	+	+	+	+	+
Steroids	+	-	+	-	+
Saponins	-	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	-	+
Alkaloids	+	-	+	+	+
Glycosides	+	+	+	+	-
Phenol	+	+	+	+	+

PL= Plantain (*Plantago lanceolata*), GL=Garlic (*Allium sativum*), IG= Ivy guard (*Coccinia grandis*), SM=Spear mint (*Mentha* spp), LG=Lemongrass (*Cymbopogon citratus*).

(+) Indicate the presence of phytochemicals and (-) Indicate the absence of phytochemicals.

Table 37. Effect of drying methods on the specific bioactive components forage herbs

Herb's name	Bioactive components	Forms	Quantity
Plantain	Acteoside	Shade dried	10.51±0.90 mg/g
		Blanching	11.23±1.00 mg/g
	Aucubin	Shade dried	20.97±2.10 mg/g
		Blanching	23.08±2.42 mg/g
Ivy-gourd	Alkaloid	Shade dried	0.149 ±0.003 mg/g
		Blanching	0.148 ±0.002 mg/g
	Saponins	Shade dried	0.121±0.001 mg/g
		Blanching	0.099±0.001 mg/g
Spear mint	Rutin	Shade dried	12.91 ±0.08 mg/g
		Blanching	10.02 ±0.01 mg/g
	Caffeic acid	Shade dried	10.97±0.04 mg/100g
		Blanching	7.98 ±0.08 mg/100g
Garlic leaves	Allicin	Shade dried	7.21 ± 0.03 mg/g
		Blanching	3.72± 0.11 mg/g
	Rutin	Shade dried	4.85± 0.05 mg/g
		Blanching	3.11 ± 0.09mg/g
Lemon grass	Limonene	Shade dried	10.10± 0.02 %
		Blanching	8.24± 0.04 %
	Citral	Shade dried	15.14± 0.06 %
		Blanching	12.16± 0.05 %

11.4.1. Sheep feeding trial using plantain and garlic leaves

a. Growth performance and digestibility

There was a difference ($P < 0.05$) in body weight gain, DM intake, CP intake, and FCR between the control and the dietary treatment groups (Table 38). The PL-, GL-, and PG-diet groups exhibited 18–26% higher daily weight gain than the CL-diet group. Similarly, higher DM and CP intake were observed in both PL- and PG-diet groups compared to the CL-diet group. Besides, the lowest FCR was observed in the PL-diet group, intermediate FCR was observed in PG- and GL-diet groups, and the highest was found in the CL-diet group. On the other hand, herbal supplementation had no effect ($P > 0.05$) on the levels of digestible crude protein (DCP), digestible crude fibre (DCF), and digestible ether extract (DEE). However, compared with the CL-diet group, the highest values for digestible nitrogen free extract (DNFE) and total digestible nutrients (TDN) were found in the PL-diet group ($P < 0.05$).

Table 38. Effect of herbal supplementation on growth performance and nutrients digestibility (g/100g DM) of sheep

Parameters		Herbal supplemented group			SEM	P value
	CL-diet	PL-diet	GL-diet	PG-diet		
Growth performance						
Initial live weight, kg	9.3±0.18	9.1±0.19	8.9±0.17	9.2±0.18	0.04	0.06
Final live weight, kg	13.1 ^c ±0.21	13.9 ^a ±0.34	13.4 ^{bc} ±0.33	13.8 ^{ab} ±0.31	0.11	0.01
Body weight gain, g/d	42.6 ^b ±3.8	53.8 ^a ±4.5	49.7 ^a ±4.9	50.6 ^a ±4.5	1.44	0.03
Dry matter intake, g/d	297 ^b ±13.8	325 ^a ±7.1	318 ^{ab} ±9.9	330 ^a ±10.3	3.04	0.02
CP intake, g/d	44.8 ^b ±1.8	48.9 ^a ±1.2	47.8 ^{ab} ±0.9	49.7 ^a ±1.2	0.39	0.02
FCR, g DM/g gain	7.6 ^a ±0.63	6.1 ^c ±0.35	6.5 ^b ±0.57	6.6 ^b ±0.57	0.15	0.04
Digestibility (g/100g DM)						
DCP	9.69±0.28	10.19±0.16	9.80±0.18	9.99±0.07	0.07	0.12
DCF	12.33±0.33	11.94±0.55	12.36±0.35	11.89±0.46	0.11	0.31
DEE	2.37±0.15	2.38±0.18	2.39±0.11	2.33±0.16	0.03	0.94
DNFE	30.40 ^b ±2.31	34.03 ^a ±0.58	30.46 ^b ±2.19	33.65 ^{ab} ±0.71	0.58	0.01
TDN	57.76 ^b ±1.77	61.53 ^a ±0.77	58.02 ^b ±1.9	60.78 ^{ab} ±0.86	0.54	0.01

DM = dry matter; CP = crude protein; FCR = feed conversion ratio; DCP = digestible crude protein; DCF = digestible crude fibre; DEE = digestible ether extract; DNFE = digestible nitrogen free extract; TDN = total digestible nutrients; CL-diet = road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellets (6 mm); PL-diet = CL-diet + 10 gDM plantain herb supplementation; GL-diet = CL-diet + 10 gDM garlic leaf supplementation; PG-diet = CL-diet + 5 gDM plantain herb and 5 gDM garlic leaf supplementation; ^{a,b,c} means in the same row with no common superscript differ significantly ($P < 0.05$)

b. Plasma metabolites, enzyme activities, and rumen histology

Plasma glucose, triglycerides, total cholesterol, and LDL were higher ($P < 0.05$) in the CL-diet group compared with all the herbal-supplemented groups (Table 39). Herbal dietary supplementation exhibited a positive effect by reducing ALT and CK values. For ALT content, the GL-diet group had higher values than the CL-diet group, and the lowest ($P < 0.05$) ALT and CK values were observed for the PL-diet group (Table 39). Besides, increased BUN values were

found in PL- and PG-diet groups, but no difference ($P > 0.05$) was observed in HDL, AST, and ALP among treatments. Rumen papillae length and width and the thickness of the tunica muscularis were improved ($P < 0.05$) in herbal-supplemented groups, whereas the highest values were observed in the PL-diet group.

Table 39. Effect of herbal supplementation on plasma metabolites, enzyme activities and rumen histology in sheep

Parameters	CL-diet	Herbal supplemented group			SE M	P value
		PL-diet	GL-diet	PG-diet		
Plasma metabolites						
Glucose (mmol/dL)	5.15 ^a ±0.09	4.58 ^b ±0.14	5.06 ^a ±0.18	4.73 ^b ±0.08	0.27	<0.001
BUN (mg/dL)	16.60 ^b ±0.86	20.56 ^a ±1.83	17.24 ^b ±0.57	19.69 ^a ±1.12	0.51	0.001
Triglycerides (mg/dL)	70.39 ^a ±2.54	58.06 ^b ±2.48	56.15 ^b ±4.85	58.41 ^b ±2.08	1.62	<0.001
Total cholesterol (mg/dL)	82.74 ^a ±4.36	74.78 ^b ±2.61	75.20 ^b ±4.59	78.73 ^{ab} ±3.14	1.18	0.038
HDL (mg/dL)	49.15±2.16	53.47±3.26	51.77±1.39	50.92±3.47	1.74	0.206
LDL (mg/dL)	20.32 ^a ±0.49	16.91 ^c ±0.83	17.55 ^{bc} ±0.69	18.46 ^b ±0.84	0.37	<0.001
Enzyme activities						
ALT (U/L)	21.57 ^a ±4.89	15.11 ^c ±3.97	21.72 ^a ±6.13	19.77 ^b ±3.93	1.07	<0.001
AST (U/L)	44.03±11.79	39.91±8.57	42.13±11.29	41.71±16.23	1.03	0.13
CK (U/L)	154.1 ^a ±39.1	121.5 ^c ±31.7	137.2 ^b ±41.3	133.7 ^b ±39.2	8.10	0.04
ALP (U/L)	179.2±57.8	196.2±78.2	197.1±69.1	181.3±61.9	17.3	0.81
Rumen histology						
Length of papillae (mm)	2.491 ^d ±0.006	2.744 ^a ±0.007	2.609 ^c ±0.005	2.732 ^b ±0.004	0.06 6	<0.001
Width of papillae (mm)	0.891 ^d ±0.004	1.085 ^a ±0.003	0.948 ^b ±0.004	0.915 ^c ±0.003	0.01 0	<0.001
Thickness of TM (mm)	1.433 ^d ±0.004	1.535 ^b ±0.003	1.471 ^c ±0.005	1.487 ^b ±0.004	0.01 1	<0.001

BUN = blood urea nitrogen; HDL = high density lipoprotein; LDL = low density lipoprotein; ALT = alanine transaminase; AST = aspartate transaminase; CK = creatine kinase; ALP = alkaline phosphatase; TM = tunica muscularis; CL-diet = road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellets (6 mm); PL-diet = CL-diet + 10 gDM plantain herb supplementation; GL-diet = CL-diet + 10 gDM garlic leaf supplementation; PG-diet = CL-diet + 5 gDM plantain herb and 5 gDM garlic leaf supplementation; ^{a,b,c} means in the same row with no common superscript differ significantly ($P < 0.05$)

c. Serum immunity and antioxidants

Increased serum albumin and globulin concentration were found in both PL- and PG-diet groups ($P < 0.05$; Table 40), and the highest ($P < 0.05$) concentrations of serum IgA, G₁, G₂, and M were found in the PL-diet group. Moreover, the sole or combined supplementation of plantain and garlic leaf had a positive effect ($P < 0.05$) on the levels of serum antioxidants (Table 40). Compared with the CL-diet group, the highest TAC, SOD, GPx, and catalase levels were found in the PL-diet group, and then in PG- and GL-diet groups.

Table 40. Effect of herbal supplementation on serum immunity and antioxidants in sheep

Parameters	CL-diet	Herbal supplemented group			SEM	P value
		PL-diet	GL-diet	PG-diet		
Serum Immunity						
Serum albumin (g/L)	31.09 ^b ±1.55	34.28 ^a ±1.42	31.25 ^b ±1.26	34.05 ^a ±0.82	0.48	0.005
Serum globulin (g/L)	21.59 ^c ±1.08	29.11 ^a ±1.21	22.62 ^c ±0.91	25.69 ^b ±0.61	0.79	<0.001
IgA (mg/mL)	0.58 ^c ±0.03	0.75 ^a ±0.04	0.64 ^b ±0.03	0.69 ^b ±0.05	0.02	<0.001
IgG ₁ (mg/mL)	17.49 ^b ±0.62	20.56 ^a ±0.36	18.02 ^b ±0.67	20.22 ^a ±0.36	0.36	<0.001
IgG ₂ (mg/mL)	6.11 ^c ±0.05	6.76 ^a ±0.04	6.19 ^c ±0.03	6.46 ^b ±0.09	0.07	<0.001
IgM (mg/mL)	1.73 ^d ±0.03	1.95 ^a ±0.04	1.79 ^c ±0.02	1.83 ^b ±0.09	0.02	<0.001
Serum Antioxidants						
TAC (μmol/L)	447.48 ^d ±5.5	494.57 ^a ±3.9	460.56 ^c ±1.1	472.29 ^b ±1.5	6.61	<0.001
GPx (μmol/L)	5.16 ^d ±0.06	5.98 ^a ±0.01	5.54 ^c ±0.07	5.75 ^b ±0.06	0.1	<0.001
SOD (U/mL)	164.19 ^c ±4.5	183.59 ^a ±1.0	172.28 ^b ±1.5	175.02 ^b ±2.5	2.71	0.01
Catalase (U/mL)	4.25 ^d ±0.08	6.36 ^a ±0.04	5.80 ^c ±0.13	6.12 ^b ±0.01	0.31	<0.001

IgA = immunoglobulin A; IgG₁ = immunoglobulin G₁; IgG₂ = immunoglobulin G₂; IgM = immunoglobulin M; TAC = total antioxidant capacity; SOD = superoxide dismutase; GPx = glutathione peroxidase; CL-diet = road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellets (6 mm); PL-diet = CL-diet + 10 gDM plantain herb supplementation; GL-diet = CL-diet + 10 gDM garlic leaf supplementation; PG-diet = CL-diet + 5 gDM plantain herb and 5 gDM garlic leaf supplementation; ^{a,b,c} means in the same row with no common superscript differ significantly ($P < 0.05$)

d. Carcass characteristics and mutton quality

There was no difference ($P > 0.05$) in carcass characteristics and mutton proximate components among the treatment groups apart from caul fat, pelvic fat, dressing percentage, rib eye area, and mutton ether extract levels (Table 41). Caul fat and pelvic fat had their lowest values in the PL-diet group followed by the PG-, GL-, and CL-diet groups. A greater dressing percentage was found in both the PL- and PG-diet groups compared with that of the other treatment groups. Furthermore, mutton ether extract content was the lowest ($P < 0.05$) and rib eye area was the highest ($P < 0.05$) in the PL-diet group. Mutton saturated fatty acids content was lower and that of unsaturated fatty acids was higher in all herbal-supplemented groups compared with the CL-diet group ($P < 0.05$; Table 41). Linoleic acid, which is of major interest for obtaining a functional meat product, was the highest in the PL-diet group followed by the PG-, GL- and CL-diet groups.

Table 41. Effect of herbal supplementation on carcass characteristics and mutton quality in sheep

Parameters	CL-diet	Herbal supplemented group			SEM	P value
		PL-diet	GL-diet	PG-diet		
Carcass parameter						
Hot carcass wt.(kg)	5.20±0.30	5.80±0.21	5.56±0.43	5.34±0.51	0.28	0.240
Fore limb (%)	0.91±0.01	0.84±0.03	0.85±0.11	0.87±0.14	0.11	0.525
Hind limb (%)	0.87±0.17	0.79±0.07	0.81±0.09	0.77±0.07	0.26	0.119
Neck (%)	2.93±0.21	3.81±0.61	3.19±0.31	3.46±0.17	0.27	0.087
Fore leg (%)	4.07±0.11	3.76±0.22	4.16±0.31	3.89±0.27	0.03	0.145
Hind leg (%)	4.09±0.31	4.78±0.17	4.54±0.24	5.65±0.28	0.05	0.117
Fat deposition						
Caul fat (%)	1.33 ^a ±0.05	0.91 ^c ±0.09	1.01 ^b ±0.07	0.97 ^{bc} ±0.04	0.09	0.036

Pelvic fat (%)	0.69 ^a ±0.06	0.30 ^c ±0.07	0.51 ^b ±0.11	0.47 ^{bc} ±0.09	1.04	<0.001
Dressing percentage (%)	40.5 ^c ±1.99	44.2 ^a ±1.64	41.5 ^b ±2.21	43.6 ^a ±1.78	0.08	<0.001
Carcass quality						
Cooking loss (%)	30.51±0.19	32.69±0.23	31.57±0.28	33.05±0.29	0.77	0.18
Drip loss (%)	4.15±0.09	4.30±0.07	4.21±0.17	4.27±0.02	0.07	0.549
Rib eye area (%)	5.05 ^c ±0.21	6.15 ^a ±0.37	5.11 ^c ±0.26	5.89 ^b ±0.17	0.27	0.012
Mutton composition						
Moisture (%)	72.63±0.04	72.52±0.01	72.64±0.01	72.62±0.06	0.06	0.08
Crude protein (%)	21.37±0.03	21.89±0.02	21.61±0.05	21.80±0.02	0.06	0.07
Ether extract (%)	4.55 ^a ±0.19	3.99 ^b ±0.04	4.08 ^b ±0.03	4.17 ^b ±0.05	0.07	0.001
Ash (%)	1.10±0.05	1.07±0.04	1.12±0.06	1.09±0.01	0.01	0.56
Nitrogen free extract (%)	72.98±0.13	73.04±0.08	73.19±0.06	72.94±0.05	0.04	0.07
Mutton fatty acid						
SFA %	50.75 ^a ±0.81	49.94 ^b ±0.62	49.98 ^b ±0.31	49.82 ^b ±0.41	0.14	<0.001
USFA %	49.25 ^b ±0.01	50.06 ^a ±0.02	50.02 ^a ±0.03	50.18 ^a ±0.04	0.17	<0.001
i) MUFA %	42.15 ^a ±0.09	40.81 ^c ±0.07	41.6 ^b ±0.13	41.7 ^b ±0.11	0.37	0.030
ii) PUFA %	7.10 ^d ±0.13	9.21 ^a ±0.04	8.42 ^c ±0.09	8.48 ^b ±0.07	0.39	0.010
Linoleic Acid (C18:2)	3.99 ^c ±0.31	5.28 ^a ±0.67	4.98 ^{bc} ±0.59	5.09 ^b ±0.51	0.21	<0.001
Linolenic Acid (C18:3)	1.18 ^a ±0.09	1.02 ^c ±0.07	1.15 ^a ±0.03	1.09 ^b ±0.04	0.03	<0.001

SFA = saturated fatty acid; USFA = unsaturated fatty acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CL-diet = road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellets (6 mm); PL-diet = CL-diet + 10 gDM plantain herb supplementation; GL-diet = CL-diet + 10 gDM garlic leaf supplementation; PG-diet = CL-diet + 5 gDM plantain herb and 5 gDM garlic leaf supplementation; ^{a,b,c} means in the same row with no common superscript differ significantly ($P < 0.05$).

11.5. Broiler feeding trial

11.5.1. Growth performance

The all-experimental treatments involved T₀, T₁, T₂ at the initial stage all bird weight were almost similar. There was no statistically significant difference of live weight of broilers at 8th, 16th, 24th days of all experimental treatments. But numerically higher live weight was found in T₁ group compared to other two dietary treatments at the 8th, 16th, 24th day. At final stage 32nd day, statistically significant higher body weight was found in T₁ group compared to the other treatments (Table 42).

Table 42. Live weight (g/bird) of broilers under different dietary treatments after 8 days interval

Age	Treatments			SEM	P-Value
	T ₀	T ₁	T ₂		
0 Days	41.33±1.52	41.33±1.52	41.00±2.00	0.98	0.962
8th Day	158.50±12.90	167.82±9.91	145.80±10.00	6.36	0.124
16th Day	479.05±11.11	494.19±6.79	462.74±10.11	5.07	0.013
24th Day	855.33±10.796	869.32±10.26	802.62±9.02	5.80	0.004
32nd Day	1612.80±13.22	1650.40±7.56	1596.20±6.50	5.52	0.001

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture,

T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant ($P < 0.05$); **Significant ($P < 0.01$); NS, Non-significant ($P > 0.05$), SEM=Standard Error Mean

11.5.2. Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) numerically lower in T1 dietary treatments that is good indicator for broiler production i.e. less feed is required for higher meat production at 8th, 16th, and 32th of age. However, T1 showed statistically significant FCR compared to other two dietary treatments at the 24th days of age (Table 43).

Table 43. Feed Conversion Ratio (g feed intake/g weight gain) of broilers under different dietary treatments after 8 days interval

Age	Treatments			SEM	P-Value
	T ₀	T ₁	T ₂		
8th Day	1.45±0.01	1.44±0.01	1.47±0.02	0.53	0.778
16th Day	1.57±0.07	1.55±0.03	1.59±0.07	13.62	0.072
24th Day	1.68±0.04	1.66±0.04	1.71±0.05	12.85	0.003
32th Day	1.82±0.04	1.79±0.01	1.85±0.02	3.14	0.955

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant (P<0.05); **Significant (P<0.01); NS, Non-significant (P>0.05), SEM=Standard Error Mean.

European Production Efficiency Factor (EPEF) involve three factors feed conversion, mortality, and daily gain, and the formula is:

$$(\text{Average grams gained/day} \times \% \text{ survival rate}) / \text{Feed Conversion} \times 10$$

Strongly significant difference was found in shade dried herb supplementation group (T₁) compared to other two dietary treatments (Table 44).

Table 44. European production efficiency factor under different dietary treatments at final stage (from 0 day to slaughtering stage)

Age	Treatments			SEM	P-Value
	T ₀	T ₁	T ₂		
Slaughtering	287.0±3.00	298.0±2.10	281.0±1.0	1.10	0.001

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture,

T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant (P<0.05); **Significant (P<0.01); NS, Non-significant (P>0.05),

SEM=Standard Error Mean

11.5.3. Meat proximate components

Proximate components of meat from different dietary treatment didn't show any statistical variation except ether extract (EE). Herbal supplementation in shade dried and blanched forced dried form exhibited the positive impact on lean meat production. Significantly (P<0.01) higher EE was found in antibiotic growth promoter fed group (T₀) (Table 45).

Table 45. Meat proximate analysis (gram/100gm) of broiler in different dietary treatments

Parameters	Treatments			SEM	P-value
	T ₀	T ₁	T ₂		
Moisture (%)	74.36±0.86	72.68±1.25	74.04±2.01	0.57	0.13
CP (%)	22.38±0.77	22.66±1.15	22.47±0.58	0.11	0.14
CF (%)	0.69±0.04	0.61±0.04	0.65±0.21	0.01	0.55
EE (%)	1.87±0.11	1.32±0.12	1.36±0.08	0.007	0.00
NFE (%)	73.84±0.84	74.10±1.31	74.41±0.65	0.11	0.41
Ash (%)	1.2±0.09	1.35±0.036	1.29±0.05	0.01	0.002

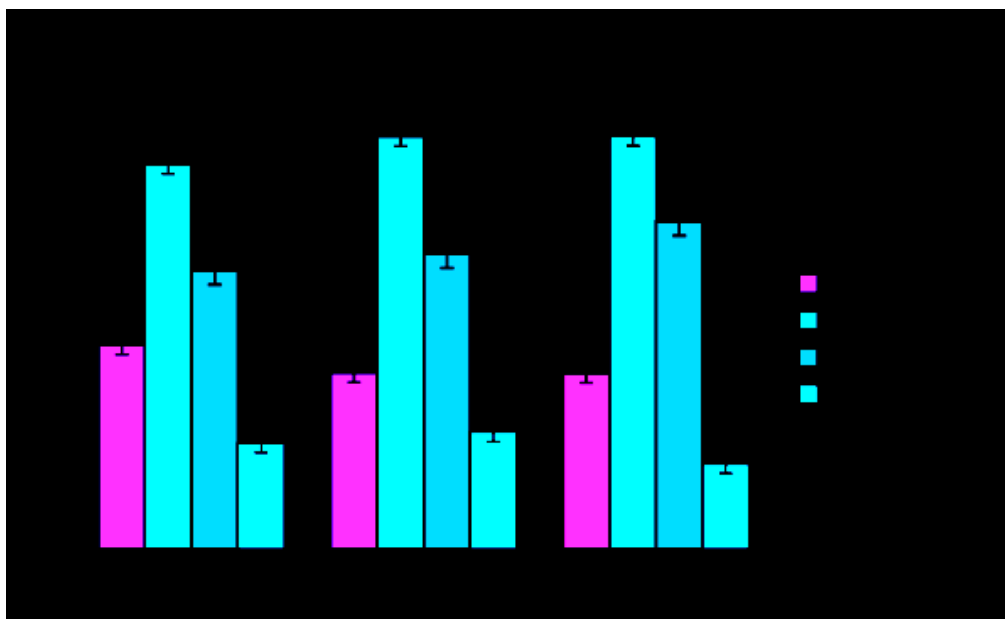
T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant (P<0.05); **Significant (P<0.01); NS, Non-significant (P>0.05), SEM=Standard Error Mean.

11.6. Meat fatty acid level in broiler meat

Numerically higher saturated fatty acid was found in antibiotic growth promoter groups compared to other two herb powder supplemented groups. Significantly higher unsaturated fatty acid was observed in shade dried herb mixture supplemented group compared to antibiotic growth promoter's supplemented group. Higher level of monounsaturated fatty acid was found in T2 group, then T1 and followed by T0 group. Higher polyunsaturated fatty acid was found in T1 group, then T0 and followed by T2 group. (Fig 18). However, in case of omega three fatty acid contents statistically significant difference was observed in T1 group compared to other two groups (Fig 19).

**Fig 18.** Effects of AGP, shade dried and blenched force dried herb mixture on meat fatty acid profile

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

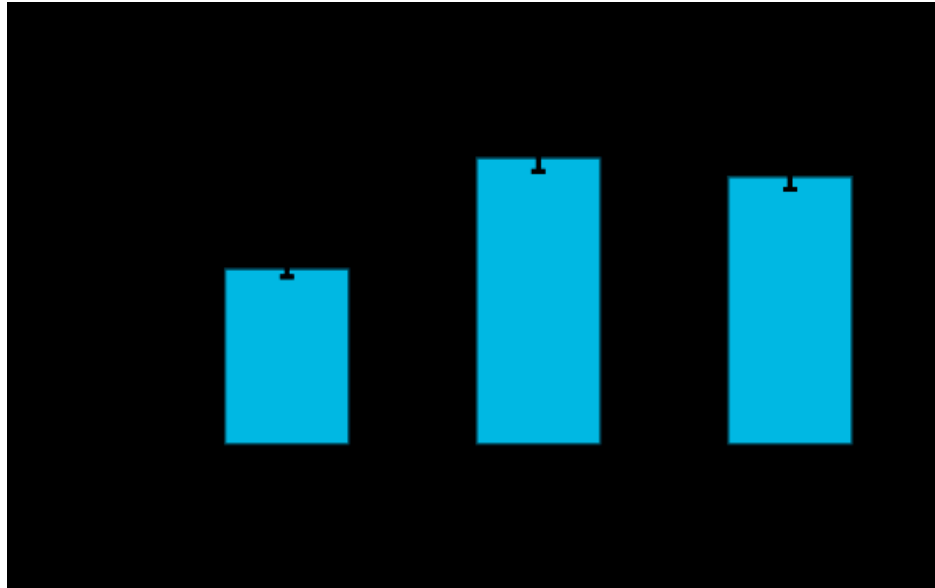


Fig 19. Effects of AGP, shade dried and blanching force dried herb mixture on omega three fatty acid

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

11.7. Shank mineralization

Shank morphology involved length significantly was observed higher in herb mixture supplemented groups compared to antibiotic fed group. Moreover, total ash content was significantly higher in herb mixture supplemented group compared to antibiotic growth promoter supplemented groups. However, numerically calcium contents of herb mixture supplementation groups were higher compared to antibiotic supplemented group (Table 46).

Table 46. Effect of AGPs, Shade dried, blenched forced dried herb mixture diets on shank mineralization of broiler birds at 32 days of age

Parameters	Treatments			SEM	P Value
	T ₀	T ₁	T ₂		
Shank Length (cm)	6.31±.01	6.65±.01	6.65±.01	0.06	0.00
Lower (cm)	1.58±.01	1.84±.01	1.58±.01	0.04	0.00
Middle (cm)	1.00±.00	1.00±.00	1.08±.11	0.02	0.46
Upper (cm)	1.68±.01	1.83±.01	1.74±.01	0.03	0.00
Total ash %	78.04±.24	80.53±.27	80.36±.42	0.44	0.00
Ca %	38.51±.70	39.50±.38	39.68±.17	0.26	0.07
P %	27.17±.23	28.39±.40	27.62±.39	0.19	0.06

T₀= Formulated diet + AGP (Control), T₁ = Formulated diet + 1.0 % (DM) Shade dried herb mixture,

T₂ = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant (P<0.05); **Significant (P<0.01); NS, Non-significant (P>0.05), SEM=Standard Error Mean

11.8. Meat colour parameters

There were no effects found on breast and thigh meat colour when herb mixture was supplied as dried forms in broilers. Significant difference was observed herb mixture supplemented groups in case of breast meat redness (Table 47). Hue angle significantly lower in herb supplemented groups (T_1 and T_2) compared to antibiotic growth promoters supplemented group (T_0). Higher saturation index was found in herb supplemented groups compared to antibiotic growth promoters supplemented group in case of breast and thigh meat. (Table 47 & 48)

Table 47. Effect of AGPs, Shade dried, blanched forced dried herb mixture diets on breast meat colour of broiler meat at 32 days of age

Parameters	Treatments			SEM	P Value
	T_0	T_1	T_2		
L	43.79±2.84	47.10±.72	46.83±1.74	0.70	0.38
a	1.77±.17	3.61±.07	3.57±.65	0.31	0.01
b	2.47±.16	3.74±.23	4.00±.86	0.25	0.11
Hue angle	54.35±4.30	45.76±1.98	48.36±.69	1.76	0.04
Saturation index	3.04±.03	5.20±.12	5.36±1.07	0.38	0.04

L= relative lightness, a= relative redness, b=relative yellowness, Higher saturation index value indicates higher meat pigmentation.

T_0 = Formulated diet + AGP (Control), T_1 = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T_2 = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant ($P<0.05$); **Significant ($P<0.01$); NS, Non-significant ($P>0.05$), SEM=Standard Error Mean

Table 48. Effect of AGPs, Shade dried, blanched forced dried herb mixture diets on thigh meat colour of broiler meat at 32 days of age

Parameters	Treatments			SEM	P value
	T_0	T_1	T_2		
L	47.64±2.60	47.09±.78	49.00±.01	0.47	0.524
a	3.48±.49	4.64±.33	4.21±.82	0.25	0.312
b	3.90±.88	3.78±.71	2.38±.08	0.29	0.189
Hue angle	47.99±2.45	39.01±3.26	29.00±4.24	2.86	0.01
Saturation index	5.22±.99	5.99±.69	4.83±.76	0.27	0.56

L= relative lightness, a= relative redness, b=relative yellowness, Higher saturation index value indicates higher meat pigmentation.

T_0 = Formulated diet + AGP (Control), T_1 = Formulated diet + 1.0 % (DM) Shade dried herb mixture, T_2 = Formulated diet + 1.0 % (DM) Blanching force dried herb mixture

Herb mixture was composed of Plantain herbs, ivy gourd, mint and garlic leaves.

*Significant ($P<0.05$); **Significant ($P<0.01$); NS, Non-significant ($P>0.05$), SEM=Standard Error Mean.

11.8.1. Layer feeding trial

Laying hen performance : The laying hen performance including egg number, egg weight, hen day egg production (HDEP), egg mass, feed intake and feed conversion ratio (FCR) was stated in Table 49. Egg number from every hen/treatment were statistically significant ($P=0.009$), ($P=0.000$), ($P=0.001$) during 32-36 week, 36-40 week and 32-40 week, respectively. During 32-36 week, addition of herb mix at 15 gkg⁻¹ diet showed better result than the other treatments and no statistical variation was obtained among 15 gkg⁻¹, 5 gkg⁻¹, and 20 gkg⁻¹ diet though supplementation of herb mix at 15 gkg⁻¹ diet showed highest number of egg/hen. Between CON

and 10 gkg⁻¹ diet group, no statistical difference was observed, and 10 gkg⁻¹ diet group produced more than 5 eggs/hen. During 36-40 week, addition of herb mix at 15 gkg⁻¹ diet obtained better result compared to CON, 10 gkg⁻¹ diet herbal supplementation and no statistical variation was observed among 15 gkg⁻¹, 10 gkg⁻¹, and 20 gkg⁻¹ diet herbs mix supplementation. Addition of 15 g herb mix kg⁻¹ diet produced more than 32, 12, 17 and 7 eggs from 36-40 weeks compared to CON, 5 gkg⁻¹, 10 gkg⁻¹, and 20 gkg⁻¹ diet. From 32-40 weeks, supplementation herbal mix showed better result compared to CON. Addition of 15 herb mix at 15 gkg⁻¹ diet obtained better number of eggs from 32-40 weeks of age, then 20 gkg⁻¹, 5 gkg⁻¹ and 10 gkg⁻¹ diet, respectively. From 32-40 weeks, herbal mix group produced more than 17, 11, 29, 21 eggs in 5 gkg⁻¹, 10 gkg⁻¹, 15 gkg⁻¹, and 20 gkg⁻¹ diet respectively, compared to CON group.

During 32-36 week, 15 g herb mix powder kg⁻¹ diet showed significant ($P=0.028$) result in terms of egg weight compared to rest groups. No statistical variation was found among herbs mix supplementation groups in terms of egg weight. CON group showed the tendency of lowest egg weight compared to herbs mix supplementation groups. From 36-40 week, herbs mix group showed the tendency of large egg compared to CON group. At 32-40 weeks, addition of herbs mixes with CON diet obtained significantly ($P=0.019$) larger eggs compared to CON group. Supplementation of herbs mix at 15 gkg⁻¹ diet produced larger size of eggs compared to 20 gkg⁻¹, 10 gkg⁻¹, 5 gkg⁻¹, and CON, respectively.

During 32-36 week age of laying hen, hen day egg production (HDEP) was significantly ($P=0.009$) higher in herbs mix groups compared to CON group. Addition of 15 g herb mix kg⁻¹ diet revealed that highest number of eggs were found in this group, and no statistical variation was observed among 15 gkg⁻¹, 5 gkg⁻¹, and 20 gkg⁻¹ diet, though 15 g herb mix kg⁻¹ diet showed the higher tendency of egg production/day. At 36-40 weeks, HDEP obtained significant ($P=0.000$) in herbal supplementation groups compared to CON. At 15 g herb mix kg⁻¹ diet obtained better result compared to other groups and no variation was observed among 15 gkg⁻¹, 20 gkg⁻¹, and 5 gkg⁻¹ diet groups. There was no statistical variation between 10 gkg⁻¹ diet and CON group, though 10 g herb mix kg⁻¹ diet produced more than 5 eggs/day. At 32-40 week, HDEP was obtained better ($P=0.001$) at herbs mix groups compared to CON group. Addition of 15 g herb mix kg⁻¹ diet showed highest number egg/hen/ day compared to CON and 10 gkg⁻¹ diet, though no statistical difference was observed among this group, 5 gkg⁻¹, and 20 gkg⁻¹ groups. Supplementation of herb mix at 10 gkg⁻¹ diet showed the better tendency compared to CON group in terms of HDEP, though HDEP was revealed that no statistical variation was observed between two groups.

Table 49. Effect of herbal mixture on laying performance from 32-40 weeks of age

Parameters	Treatments					SEM	P- value
	CON	CON+5g HM/kg diet	CON+10g HM/kg diet	CON+15g HM/kg diet	CON+20g HM/kg diet		
Egg number/hen/group at first 1-28 days							
Week 32-36	290.4 ^b ±14.06	302.8 ^{ab} ±7.79	295.8 ^{b0} ±5.54	316.0 ^a ±3.44	306.0 ^{ab} ±16.07	2.66	0.009
Week 36-40	285.20 ^c ±9.81	305.60 ^{ab} ±6.99	300.60 ^{bc} ±6.42	317.0 ^a ±7.42	310.2 ^{ab} ±10.52	2.67	0.000
Week 32-40	287.80 ^{bc} ±11.76	304.20 ^{abc} ±6.68	298.20 ^{bc} ±5.54	316.80 ^a ±4.02	308.40 ^{ab} ±13.12	2.58	0.001
Egg weight (g)							
Week 32-36	62.09 ^b ±0.76	63.80 ^{ab} ±1.96	63.95 ^{ab} ±1.24	65.23 ^a ±1.21	63.18 ^{ab} ±1.52	0.33	0.028
Week 36-40	62.69 ^b ±1.20	64.80 ^{ab} ±2.01	64.95 ^{ab} ±1.22	66.03 ^a ±1.49	64.37 ^{ab} ±1.95	0.37	0.051

Week 32-40	62.39 ^b ±0.56	64.30 ^{ab} ±1.95	64.45 ^{ab} ±1.18	65.63 ^a ±1.18	63.77 ^{ab} ±1.50	0.33	0.019
HDEP, %							
Week 32-36	86.43 ^b ±4.18	90.12 ^{ab} ±2.31	88.04 ^b ±1.65	94.23 ^a ±1.02	91.25 ^{ab} ±4.78	0.79	0.009
Week 36-40	84.88 ^c ±2.91	90.95 ^{ab} ±2.08	89.46 ^{bc} ±1.91	94.35 ^a ±2.21	92.32 ^{ab} ±3.13	0.80	0.000
Week 32-40	85.65 ^c ±3.50	90.53 ^{ab} ±1.98	88.75 ^{bc} ±1.64	94.28 ^a ±1.20	91.79 ^{ab} ±3.90	0.76	0.001
Egg mass (g/h/d)							
Week 32-36	53.67 ^b ±2.96	57.51 ^b ±2.73	56.29 ^b ±1.23	61.46 ^a ±0.87	57.59 ^{ab} ±1.80	0.64	0.000
Week 36-40	53.22 ^b ±2.34	58.93 ^a ±2.18	58.10 ^a ±1.26	62.35 ^a ±2.86	59.43 ^a ±2.68	0.74	0.000
Week 32-40	53.45 ^c ±2.48	58.22 ^{ab} ±2.34	57.19 ^{bc} ±1.14	61.89 ^a ±1.72	58.51 ^{ab} ±2.02	0.66	0.000
Feed intake (g/h/d)							
Week 32-36	113.49 ^b ±3.09	116.43 ^{ab} ±3.85	113.59 ^b ±2.39	120.60 ^a ±1.43	116.24 ^{ab} ±4.92	0.81	0.021
Week 36-40	115.10 ^b ±5.05	120.0 ^{ab} ±3.88	116.57 ^{ab} ±0.59	122.98 ^a ±3.03	119.22 ^{ab} ±3.98	0.87	0.023
Week 32-40	114.29 ^b ±3.54	118.21 ^{ab} ±3.81	115.08 ^b ±1.39	121.79 ^a ±1.36	117.73 ^{ab} ±4.35	0.78	0.010
FCR (egg basis)							
Week 32-36	2.12 ^a ±0.06	2.02 ^b ±0.03	2.02 ^b ±0.01	1.96 ^b ±0.04	2.01 ^b ±0.03	0.01	0.000
Week 36-40	2.16 ^a ±0.09	2.03 ^b ±0.01	2.00 ^b ±0.03	1.97 ^b ±0.04	2.00 ^b ±0.05	0.02	0.000
Week 32-40	2.14 ^a ±0.06	2.03 ^b ±0.02	2.01 ^b ±0.02	1.96 ^b ±0.03	2.01 ^b ±0.03	0.01	0.000

CON: Control diet contained corn soya based ingredients and having 17.47% CP and 2770.9 Kcal ME/Kg DM, **HM:** Herb mixture was composed of plantain herbs, ivy gourd, mint and garlic leaves, **HDEP:** Hen day egg production, **FCR:** Feed conversion ratio, **g:** Gram, **h:** Hen, **d:** Day, *Significant (P<0.05); **Significant (P<0.01) ***Highly significant (P<0.000); NS, Non-significant (P>0.05), SEM=Standard Error of Mean

Egg mass was significantly (P=0.000) better in herbs mix supplemented groups compared to CON group during 32-36, 36-40 and 32-40 week age of laying hen. During 32-36 week, CON group showed lowest egg mass. Then 10 gkg⁻¹ diet, followed by 5 gkg⁻¹, 20 gkg⁻¹ and 15 gkg⁻¹ diet groups showed the tendency to increase the egg mass, respectively. During 36-40 week, addition of herbs mix with CON diet showed the significantly better result compared to CON group, and highest egg mass was obtained at 15 gkg⁻¹, 20 gkg⁻¹, 5 gkg⁻¹ and 10 gkg⁻¹ diet groups, respectively. During 32-40 week, incorporation of herbs mix at 15 gkg⁻¹ diet increased the egg mass compared to CON and 10 gkg⁻¹ diet groups, and no statistical variation was found among this group and 5 gkg⁻¹, 20 gkg⁻¹ diet groups.

Feed intake was significant (P=0.021) between 15 gkg⁻¹, 5 gkg⁻¹ diet groups, and 10 gkg⁻¹ diet and CON groups at 32-36 week. No statistical difference was observed among 15 gkg⁻¹, 5 gkg⁻¹, 20 gkg⁻¹ diet groups, though 15 gkg⁻¹ diet group took highest amount of feed. Feed intake was more in 10 gkg⁻¹ diet group compared to CON group, though no statistical variation was obtained between two groups. During 36-40 week, feed intake was found in highest in 15 gkg⁻¹ diet group, then 5 gkg⁻¹, followed by 20 gkg⁻¹, and 10 gkg⁻¹ diet groups and statistical difference was observed among 5 gkg⁻¹, 20 gkg⁻¹, and 10 gkg⁻¹ diet groups. During 32-40 week, incorporation of herbs mix with CON group significantly (P=0.010) increased the feed intake consumption compared to CON group. At 5 g herbs mix kg⁻¹ diet with CON showed better feed intake and no statistical variation was obtained between 5 gkg⁻¹ diet and CON groups. Higher feed consumption was observed in 15 gkg⁻¹, then 5 gkg⁻¹, and 20 gkg⁻¹ diet herbs mix supplemented groups and significant variation was observed among these groups.

Feed conversion ratio significantly (P=0.000) better in herbs mix supplemented groups compared to CON group, during 32–36-week, 36-40 week, and 32-40 week age of laying hen. During 32–36-week, better FCR was calculated in 15 gkg⁻¹, then 20 gkg⁻¹, 10 gkg⁻¹, and 5 gkg⁻¹ diet groups, respectively, and no statistical difference was observed among these groups. During 36-40 week, addition of herbs mix at 15 gkg⁻¹, 10 gkg⁻¹, 20 gkg⁻¹, and 5 gkg⁻¹ diet with CON showed that

significantly reduced the FCR that was better for layer industry, respectively. At 32-40 week, more FCR was calculated in CON group that was significant compared to all herbs mix supplemented groups. Better FCR was calculated at 15 gkg⁻¹, then 10 gkg⁻¹, 20 gkg⁻¹ and 5 gkg⁻¹ diet herbs mix supplemented groups, respectively.

11.9. Egg external quality at the end of 36 week of age

As shown in Table 50, addition of herbs mixes at 5 gkg⁻¹, 10 gkg⁻¹, 15 gkg⁻¹, and 20 gkg⁻¹ diet with CON had no significant effects in term of egg diameter (P=0.598), egg surface area (P=0.330), shell thickness (P=0.529), shell weight (P=0.524) and shell percent (P=0.329). Herbs mix supplementation showed the tendency of improvement the egg length (P=0.044) and egg shape index (P=0.050) compared to CON group and no statistical difference was observed due to herbs mix supplementation.

11.10. Egg internal quality at the end of 36 week of age

Egg internal quality including albumen height, albumen diameter, albumen index, yolk height, yolk diameter, yolk index, Haugh unit and yolk colour of at the end of 36-week age of laying hen were stated in Table 50. Herbs mix supplementation at different level like 5, 10, 15 and 20 gkg⁻¹ diet had no positive effect on egg internal quality except yolk colour. Yolk colour significantly (P=0.001) improved in herbs mix supplemented groups compared to CON group. Addition of 10 g herb mix kg⁻¹ diet showed more yellow yolk colour, then 15 gkg⁻¹, 5 gkg⁻¹, and 20 gkg⁻¹ g diet groups, respectively (Table 50).

11.11. Egg external quality at the end of 40 week of age

Egg external quality including egg diameter, egg length, egg shape index, egg surface area, shell thickness, shell weight and shell percent were measured at the end of 40 week of age that were shown in Table 50. Incorporation of herbs mix at the 5, 10, 15 and 20 gkg⁻¹ diet had no significant effects on egg external quality. Herbal supplementation showed the tendency to improve the egg shape index, egg surface area, and shell thickness of egg (Table 50).

Table 50. Effect of herbal mixture on external and internal quality of egg at 36th and 40th week of age

Parameters	Treatments					SEM	P- value
	CON	CON+5g HM/kg diet	CON+10g HM/kg diet	CON+15g HM/kg diet	CON+20g HM/kg diet		
At the end of 36 week of age							
Sample egg weight (g)	62.91±6.38	61.83±3.05	63.66±6.48	65.48±2.53	66.00±2.58	0.73	0.344
Egg external quality							
Egg diameter (cm)	4.49±0.23	4.42±0.07	4.42±0.11	4.45±0.09	4.51±0.06	0.02	0.598
Egg length (cm)	5.76 ^{ab} ±0.35	5.53 ^b ±0.08	5.71 ^{ab} ±0.11	5.81 ^{ab} ±0.07	5.70 ^{ab} ±0.12	0.03	0.044
Egg shape index, (%)	78.07 ^{ab} ±3.49	79.89±0.87	77.52 ^{ab} ±2.65	76.64 ^b ±1.68	79.06 ^{ab} ±1.35	0.38	0.050
Egg surface	73.86±5.30	73.05±2.54	74.49±5.24	76.05±2.08	76.47±2.12	0.60	0.330

Parameters	Treatments					SEM	P-value
	CON	CON+5g HM/kg diet	CON+10g HM/kg diet	CON+15g HM/kg diet	CON+20g HM/kg diet		
area(cm ³)							
Shell thickness (mm)	0.34±0.02	0.33±0.02	0.33±0.01	0.35±0.03	0.34±0.03	0.004	0.529
Shell weight (g)	6.93±0.81	7.13±0.65	7.35±0.53	7.06±0.54	7.35±0.23	0.09	0.524
Shell, (%)	11.00±0.63	11.53±0.96	11.61±1.21	10.87±0.97	11.16±0.50	0.14	0.329
Egg internal quality							
Albumen height (cm)	0.75±0.05	0.74±0.05	0.68±0.06	0.76±0.08	0.71±0.08	0.01	0.137
Albumen diameter (cm)	7.64±0.71	7.53±0.33	7.66±0.31	7.65±0.31	7.83±0.68	0.07	0.822
Albumen index (%)	9.90±1.18	9.94±0.86	8.83±0.85	9.92±1.38	9.24±1.80	0.20	0.296
Yolk height (cm)	1.75±0.06	1.76±0.09	1.74±0.05	1.75±0.05	1.76±0.07	0.01	0.996
Yolk diameter (cm)	4.08±0.13	4.12±0.14	4.10±0.14	4.05±0.13	4.12±0.14	0.02	0.784
Yolk index (%)	43.00±2.34	42.56±2.35	42.45±1.56	43.29±2.31	52.57±1.93	0.32	0.921
Haugh Unit	85.85±4.17	85.93±3.54	80.87±3.94	85.27±3.94	82.53±5.55	0.76	0.119
Yolk colour	5.25 ^{ab} ±0.71	6.25 ^a ±0.71	6.62 ^a ±0.52	6.34 ^a ±0.52	6.13 ^{ab} ±0.64	0.12	0.001
At the end of 40 week of age							
Sample egg weight (g)	63.74±10.74	61.65±3.72	63.08±5.02	65.54±4.39	63.82±3.28	0.93	0.788
Egg external quality							
Egg diameter (cm)	4.35±0.18	4.42±0.10	4.45±0.15	4.47±0.13	4.43±0.07	0.02	0.436
Egg length (cm)	5.53±0.27	5.60±0.15	5.58±0.27	5.76±0.19	5.76±0.16	0.03	0.128
Egg shape index, (%)	78.84±3.69	78.90±2.92	79.84±4.48	77.72±2.71	76.90±2.01	0.52	0.435
Egg surface area(cm ³)	74.44±8.74	72.86±3.12	74.04±4.15	76.07±3.58	74.67±2.71	0.76	0.783
Shell thickness (mm)	0.34±0.03	0.35±0.04	0.34±0.02	0.34±0.01	0.34±0.03	0.004	0.892
Shell weight (g)	7.26±0.68	7.18±0.61	7.00±0.40	7.31±0.25	7.36±0.31	0.07	0.591
Shell, (%)	11.55±1.35	11.66±0.94	11.13±0.83	11.19±0.56	11.56±0.77	0.14	0.717
Egg internal quality							
Albumen height (cm)	0.74±0.08	0.72±0.06	0.68±0.07	0.76±0.08	0.71±0.12	0.01	0.485
Albumen diameter (cm)	7.49±0.49	7.61±0.39	7.75±0.47	7.33±0.39	7.84±0.43	0.07	0.170
Albumen index (%)	9.96±1.65	9.51±1.02	8.94±1.37	10.49±1.35	9.24±2.10	0.25	0.303
Yolk height (cm)	1.69±0.04	1.69±0.06	1.75±0.13	1.79±0.11	1.72±0.05	0.01	0.185
Yolk diameter (cm)	3.91 ^b ±0.16	4.15 ^a ±0.06	4.12 ^{ab} ±0.25	4.13 ^{ab} ±0.16	4.08 ^{ab} ±0.15	0.03	0.043
Yolk index (%)	43.55±2.38	40.81±1.67	42.55±4.16	43.41±3.23	42.23±2.18	0.46	0.334
Haugh Unit	84.78±6.44	84.36±4.26	81.79±5.04	85.98±4.60	83.04±7.78	0.89	0.645

Parameters	Treatments					SEM	P-value
	CON	CON+5g HM/kg diet	CON+10g HM/kg diet	CON+15g HM/kg diet	CON+20g HM/kg diet		
Yolk colour	5.13 $\pm$ 0.64	5.88 ^{ab} $\pm$ 0.64	6.00 $\pm$ 0.53	6.12 $\pm$ 0.35	6.00 $\pm$ 0.53	0.10	0.007

CON: Control diet contained corn soya based ingredients and having 17.47% CP and 2770.9 Kcal ME/Kg DM, HM: Herb mixture was composed of plantain herbs, ivy gourd, mint and garlic leaves, HDEP: Hen day egg production, FCR: Feed conversion ratio, g: Gram, cm: Centimetre, *Significant (P<0.05); **Significant (P<0.01) ***Highly significant (P<0.000); NS, Non-significant (P>0.05), SEM=Standard Error of Mean.

11.12. Egg internal quality at the end of 40 week of age

In Table 50, the egg internal quality was presented including the albumen height, albumen diameter, albumen index, yolk height, yolk index, Haugh unit and yolk colour. There was no effect on herbs mix supplementation on albumen height, diameter, and index. Besides addition of herbs mix at 5, 10, 15, and 20 gkg⁻¹ diet had no significant effects on yolk height, index and Haugh unit compared to CON group. Yolk diameter significantly (P=0.043) increased in herbs mix supplemented groups compared to CON group and better result was obtained at 5 gkg⁻¹ and then 15 gkg⁻¹ diet groups. Addition of 15 g herbs mix kg⁻¹ diet showed the higher tendency of Haugh unit compared to other herbs mix groups. Yolk colour was significantly (P=0.007) ameliorated in herbs mix supplemented groups compared to CON group. No statistical variation was observed between 5 gkg⁻¹ diet, and CON groups. Better yolk colour was obtained in 15, then 10, and 20 gkg⁻¹ diet groups, respectively (Table 50).

11.13. Dairy cow feeding trial (Expt-1 using plantain)

a. Milk yield

Plantain herb powder (PHP) supplementation at 50, 100 and 150 gram/animal increased the milk where better result was obtained at PHP100 and PHP150 group compared to control group (Table 51).

Table 51. Effects of different level of plantain herb powder (PHP) supplementation milk production and DM intake (Mean $\pm$ SE)

Parameters	Treatments				SEM	P-value
	PHP0	PHP50	PHP100	PHP150		
DMI (kg for 56 days)	797.35 $\pm$ 72	766.13 $\pm$ 73	800.80 $\pm$ 23	901.18 $\pm$ 41	0.127	0.034
DM intake (kg/day)	14.24 $\pm$ 1.2	13.68 $\pm$ 1.31	14.30 $\pm$ 0.42	16.09 $\pm$ 0.73	0.03	0.032
MY (kg for 56 days)	452.69 $\pm$ 46	454.87 $\pm$ 31	474.51 $\pm$ 20	569.14 $\pm$ 38	0.02	0.013
MY (kg/day)	8.0 $\pm$ 2.09	8.12 $\pm$ 0.55	8.47 $\pm$ 0.37	10.16 $\pm$ 2.4	0.11	0.001

PHP: Plantain herb powder; DMI: Dry matter intake; DM: Dry matter; MY: Milk yield

b. Milk composition

There is no effect of PHP on milk composition except milk protein percentage. Highest amount of protein was obtained in PHP100 group compared to PHP0, PHP50 and PHP150 (Table 52).

Table 52. Effects of different level of plantain herb powder (PHP) supplementation on milk composition (Mean $\pm$ SE)

Parameters (%)	Treatments				SEM	P-value
	PHP0	PHP50	PHP100	PHP150		
TS	12.13 $\pm$ 0.22	12.31 $\pm$ 0.17	11.31 $\pm$ 0.78	10.25 $\pm$ 0.76	0.123	0.762
SNF	7.49 $\pm$ 0.37	7.60 $\pm$ 0.03	7.66 $\pm$ 0.73	7.03 $\pm$ 0.26	0.765	0.541
Fat	4.63 $\pm$ 0.31	4.71 $\pm$ 0.20	3.65 $\pm$ 0.22	3.22 $\pm$ 0.61	0.061	0.031
Protein	2.80 $\pm$ 0.17	2.79 $\pm$ 0.01	3.08 $\pm$ 0.21	2.58 $\pm$ 0.07	0.03	0.068
Minerals	0.59 $\pm$ 0.01	0.61 $\pm$ 0.01	0.62 $\pm$ 0.03	0.57 $\pm$ 0.05	0.065	0.563

PHP: Plantain herb powder; TS: Total solids; SNF: Solids-not-fat

As presented in table 8 there is no effect of PHP on milk composition except milk fat percentage.

c. *In-vivo* digestibility of diet in dairy cows

Plantain herbal powder (PHP) supplementation improves the digestibility of dairy cows compared to control (PHP0) group (Fig 20). PHP100 showed the highest digestibility in terms of dry matter (DM) and crude protein (CP), whereas highest crude fibre (CF) digestibility was obtained at PHP150 group.

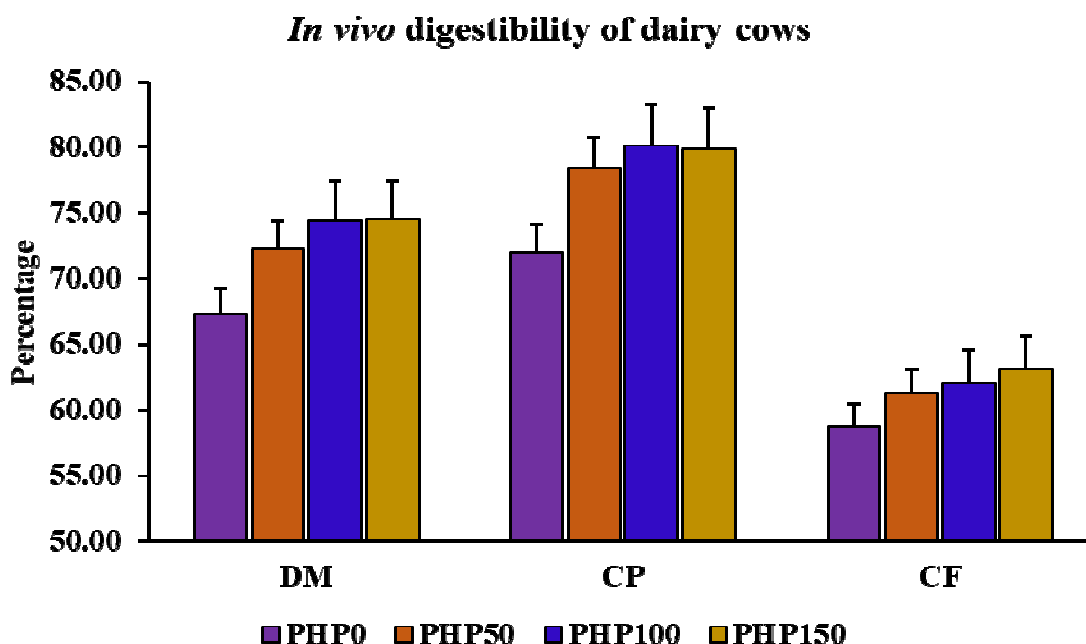


Fig 20. Effects of different level of plantain herb powder (PHP) supplementation on digestibility of offered feed in dairy cow.

d. Milk quality

Plantain herb powder (PHP) supplementation improves the milk quality compared to control group or PHP0 (Table 53 and Fig 21). The DPPH level in milk was obtained higher in PHP100, PHP150 and PHP50 group respectively compared to PHP0. Addition of PHP increased the β -

carotene content in milk and highest level was obtained at PHP150, PHP100 and PHP50 groups. The milk immunoglobulin was raised due to PHP supplementation. Milk IgG, IgM and IgG were increased in PHP100, PHP50 and PHP150 group compared to PHP50.

Table 53. Effects of different level of plantain herb powder (PHP) supplementation on milk quality (Mean $\pm$ SE) of dairy cows

Parameters	Treatments				SEM	P-value
	PHP0	PHP50	PHP100	PHP150		
DPPH, %	4.867 $\pm$ 0.30	6.006 $\pm$ 0.11	7.015 $\pm$ 0.58	5.970 $\pm$ 0.18	0.056	0.023
β -carotene	25.04 $\pm$ 2.07	28.22 $\pm$ 1.56	30.89 $\pm$ 0.88	32.28 $\pm$ 0.91	0.479	0.001
IgG, (g/L)	0.1749 $\pm$ 0.005	0.1946 $\pm$ 0.012	0.2046 $\pm$ 0.014	0.1950 $\pm$ 0.012	0.036	0.004
IgM, (g/L)	0.0449 $\pm$ 0.009	0.0395 $\pm$ 0.002	0.0395 $\pm$ 0.002	0.0385 $\pm$ 0.002	0.067	0.065
IgA, (g/L)	0.2640 $\pm$ 0.022	0.3107 $\pm$ 0.011	0.3371 $\pm$ 0.013	0.3372 $\pm$ 0.013	0.053	0.049

e. Milk fatty acid profile

Plantain herb powder (PHP) has the potentiality to improve the fatty acid profile. The milk fatty acid profile was significantly improved in PHP supplementation groups compared to control group. The saturated fatty acid level (SFA) was reduced and unsaturated fatty acid level (USFA) was raised in PHP50, PHP100 and PHP150 group and optimum result was obtained at PHP100 group.

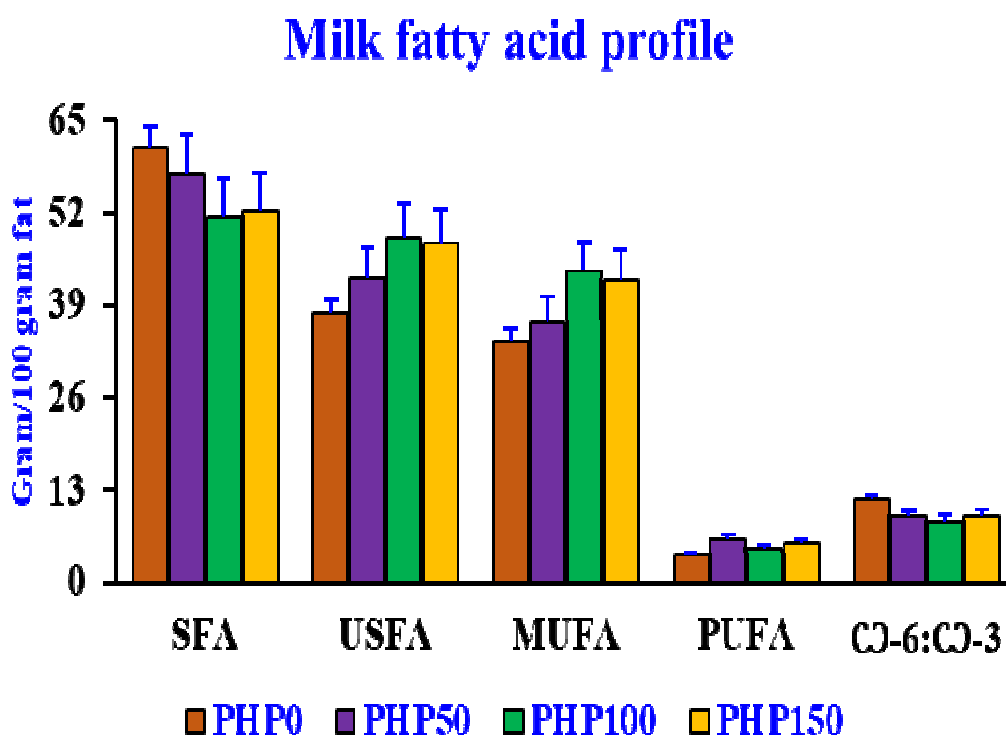


Fig 21. Effects of different level of plantain herb powder (PHP) supplementation on milk fatty acid profile of dairy cow.

11.14. Dairy cow trial (Expt-2) using plantain and lemongrass

a. Nutrient digestibility

The nutrients digestibility of Holstein crossbred cows offered control diet (CL-diet) with solely plantain herb powder (PT-D), or lemongrass powder (LG-D) and combination of plantain-lemongrass powder (PL-D) groups are presented in Table 54. The herbal supplementation groups (PT-D, LG-D, and PL-D) showed the tendency of higher nutrients intake compared to CL-diet. DM, OM, CP, NDF, ADF and EE intake were significantly ($P=0.023$) higher in PT-D group. Besides, herbal supplementation were showed the tendency of less nutrients losses through faeces except crude protein. CP excretion through faeces were obtained higher at herbal supplementation groups compared to CL-D. Statistically ($P=0.001$) and approximately 10% more CP excretion through faeces were obtained at PT-D compared to CL-diet.

Ether extract (EE) excretion significantly ($P=0.003$) obtained lower in herbal supplementation groups compared to CL-D group. LG-D and PL-D groups significantly ($P=0.005$) showed lowest minerals excretion.

The herbal supplementation groups significantly improved the nutrients digestibility (except ADF) of diet compared to CL-D group. DM ($P=0.023$), OM ($P=0.050$), CF ($P=0.009$), NDF ($P=0.007$), EE ($P<0.001$), and Ash ($P=0.018$) digestibility of diet significantly improved in herbal supplementation groups compared to CL-D group. Approximately 5.0, 3.0, 4.6% DM digestibility were obtained higher at PT-D, LG-D and PL-D groups respectively, compared to CL-D group. Digestibility of OM were significantly influenced by herbal supplementation and highest value was obtained at PT-D, PL-D and LG-D groups and no statistical variation was observed among the herbal powder supplementation groups. The herbal supplementation significantly ($P=0.006$) showed the lowest tendency of CP digestibility compared to CL-D group. CP digestibility was obtained lowest at PT-D, PL-D, LG-D, CL-D group, respectively. Significantly higher CF and NDF digestibility were obtained at PT-D, PL-D and LG-D group respectively, compared to CL-diet group, whereas no statistical variation was found between PT-D and PL-D groups. EE digestibility in Holstein crossbred dairy cows were obtained 6.5% higher at PL-D and 5.5% PT-D and LG-D groups compared to CL-D group. The herbal supplementation significantly influenced the minerals digestibility compared to CL-diet group and better value was obtained at PL-D, LG-D and PT-D groups, respectively and no statistical variation was found among the herbal supplementation groups.

Table 54. Nutrients digestibility (mean± SE) of dairy cows offered herbal supplementation

Parameters	Diets				SEM	P-Value
	CL-D	PT-D	LG-D	PL-D		
Nutrients intake (kg/day)						
Fresh ration	42.27±0.64	46.83±0.67	43.42±0.63	44.11±0.64	0.299	0.023
DM	13.19±0.20	14.62±0.21	13.55±0.20	13.77±0.20	0.093	0.023
OM	11.92±0.18	13.20±0.19	12.24±0.18	12.44±0.18	0.084	0.023
CP	1.98±0.03	2.19±0.03	2.03±0.03	2.06±0.03	.014	0.023
CF	2.94±0.05	3.26±0.05	3.02±0.04	3.07±0.05	0.021	0.023
-NDF	1.41±0.02	1.57±0.02	1.45±0.02	1.47±0.02	0.010	0.023
-ADF	0.94±0.01	1.04±0.02	0.97±0.01	0.98±0.01	0.007	0.023
EE	0.62±0.01	0.69±0.01	0.64±0.01	0.65±0.01	0.004	0.023
Ash	1.28±0.02	1.41±0.02	1.31±0.02	1.33±0.02	0.009	0.023
Nutrients excretion (kg/day)						
DM	3.60±0.10	3.46±0.11	3.39±0.10	3.30±0.11	0.049	0.324
OM	3.04±0.11	2.89±0.11	2.88±0.11	2.80±0.11	0.050	0.525
CP	0.44±0.01	0.56±0.01	0.45±0.01	0.46±0.01	0.004	0.001
CF	1.01±0.03	0.92±0.03	0.97±0.03	0.92±0.03	0.015	0.255
-NDF	0.56±0.02	0.53±0.02	0.53±0.02	0.52±0.02	0.008	0.416
-ADF	0.45±0.02	0.45±0.02	0.45±0.02	0.43±0.02	0.007	0.713
EE	0.13±0.003	0.12±0.003	0.11±0.003	0.11±0.003	0.001	0.003
Ash	0.56±0.01	0.57±0.01	0.52±0.01	0.49±0.01	0.004	0.005
Nutrients digestibility (%)						
DM	72.73±0.57	76.38±0.60	74.94±0.57	76.06±0.57	0.268	0.023
OM	74.54±0.66	78.14±0.69	76.49±0.65	77.47±0.66	0.308	0.050
CP	78.01±0.41	74.51±0.43	77.88±0.40	76.88±0.41	0.191	0.006
CF	65.58±0.73	71.75±0.77	68.04±0.72	69.95±0.73	0.340	0.009
-NDF	60.45±0.65	66.36±0.69	63.57±0.64	64.79±0.65	0.305	0.007
-ADF	51.78±1.18	56.75±1.25	53.48±1.17	56.11±1.19	0.553	0.094
EE	78.58±0.28	82.87±0.29	82.87±0.27	83.68±0.28	0.129	<0.001
Ash	55.76±0.99	59.99±1.04	60.42±0.98	62.91±0.99	0.463	0.018

CL-D: German grass, broken rice, wheat bran, mustard oil cake, DCP, and salt; PT-D: CL-D + 100 g plantain powder/day/cow; LG-D: CL-D+100g lemongrass powder/day/cow; PL-D: CL-D + 50 g plantain and 50 g lemon grass powder, SEM: standard error of mean, P<0.05 significant

b. Milk yield

Milk yield, energy corrected, and fat corrected milk yield significantly improved through herbal supplementation, and dry matter intake was also higher in supplemented group (Table 55). Total solid, SNF, lactose and protein contents were improved in supplemented group and LG-D found better compared to PT-D. Milk fat was numerically lower in PT-D compared to CL-D and LG-D however no variation was observed between CL-D and LG-D.

Table 55. Milk production performances (mean± SE) of dairy cows offered herbal supplementation

Parameters	Groups				SEM	P-value or Effects		
	CL-D	PT-D	LG-D	PL-D		D	T	D × T
Milk performance (kg/d)								
DMI	12.91±0.09	13.70±0.10	13.49±0.09	13.28±0.09	0.043	<0.001	0.001	0.862
MY	7.35±0.32	8.81±0.34	9.08±0.32	7.98±0.32	0.150	0.002	0.092	0.916
ECM	7.02±0.27	8.22±0.28	8.87±0.27	7.79±0.27	0.126	<0.001	0.001	0.488
FCM	4.42±0.18	4.87±0.19	5.17± 0.18	4.70±0.18	0.083	0.038	<0.001	0.460
FE (DMI/ ECM)	1.87±0.07	1.80±0.07	1.67± 0.07	1.78± 0.07	0.032	0.227	0.233	0.958
Milk component yield (g/d)								
TS	879±34.00	1047±36.00	1128±35.00	987±35.00	16.00	<0.001	0.003	0.524
SNF	584.76±25.45	723.08±27.12	783.58±25.86	673.70±25.79	12.05	<0.001	0.020	0.619
Lactose	300.40±12.74	365.64±13.57	383.02±12.94	343.05±12.91	6.031	0.001	0.016	0.487
Protein	239.78±11.94	303.26±12.73	344.26±12.14	280.79±12.10	5.656	<0.001	0.046	0.799
Fat	294.38±11.64	324.37±12.40	344.31±11.82	313.26±11.79	5.510	0.039	<0.001	0.460
Milk composition (%)								
TS	12.02±0.21	12.23±0.23	12.79±0.22	12.27±0.21	0.100	0.101	0.040	0.636
SNF	8.00±0.12	8.34±0.13	8.74±0.12	8.33±0.12	0.057	0.002	0.191	0.176
Lactose	4.10±0.08	4.21±0.09	4.30±0.09	4.26±0.09	0.040	0.392	0.316	0.228
Protein	3.30±0.04	3.50±0.04	3.80±0.04	3.45±0.04	0.016	<0.001	0.044	0.103
Fat	4.02±0.13	3.89±0.13	4.01±0.13	3.94±0.13	0.060	0.067	0.025	0.936
pH	6.57±0.03	6.91±0.03	6.84±0.03	6.79±0.03	0.012	<0.001	<0.001	<0.001
E. con	7.24±0.12	7.35±0.13	7.54±0.1	7.32±0.12	0.057	0.401	0.429	0.506

CL-D: German grass, broken rice, wheat bran, mustard oil cake, DCP, and salt; PT-D: CL-D + 100 g plantain powder/day/cow, LG-D: CL-D + 100g lemongrass powder/day/cow; PL-D: CL-D + 50 g plantain and 50 g lemon grass powder, DMI: dry matter intake, MY: milk yield, ECM: energy corrected milk, FCM: fat corrected milk, FE: feed efficiency, TS: total solids, SNF: solids not fat, E. con: electrical conductivity. $p < 0.05$, SEM: standard error of mean, D: Diet, T: Time

Covariates (PN, DIM and BW) appearing in the model are evaluated at the following values: PN = 2.50, DIM = 105.00, BW = 430.3192.

c. Blood biomarkers, antioxidants, minerals and immune status

The data related to blood biomarkers are represented in Table 56. Supplementation of plantain (PT-D), lemon (LG-D) and plantain-lemon powder (PL-D) with control diet (CL-D) in dairy cows positively influence the serum lipid profile. Compared to CL-D, the serum triglycerides in dairy cows were significantly reduced due to herbal supplementation and better value was obtained at LG-D, PL-D and PT-D groups. The serum cholesterol (TC) level of dairy cows was positively influenced by herbal supplementation (D), time (T) and interaction between diet and time (D×T) ($P < 0.001$). Better serum TC was obtained at PT-D, PL-D and LG-D and no significant variation was found between LG-D and PL-D groups. Compared to CL-D, serum good cholesterol (HDL-C) and bad cholesterol (LDL-C) level was significantly increased and decreased due to D, T and D×T, respectively ($P < 0.001$). The PT-D significantly improved the level of HDL-C, whereas the LDL-C level was significantly reduced at PT-D, PL-D and LG-D respectively, compared to CL-D. The serum VLDL-C was not influenced by time and diet-time interaction, while the diet showed positive influence and better value was obtained at LG-D, PL-D and PT-D, respectively, compared to CL-D.

The serum total antioxidant capacity (TAC) in Holstein crossbred cows were influenced by herbal diets, time ($P<0.001$) and diet-time interaction ($D \times T$) ($P<0.05$). Compared to CL-D, 5.4, 4.73 and 4.5% higher serum TAC was obtained at PL-D, LG-D and PT-D groups and no statistical variation was observed among the herbal diets. Serum SOD level in Holstein dairy cows were positively influenced by herbal diets ($P<0.001$), and time ($P<0.05$) and higher value was obtained at LG-D, PT-D and PL-D groups, respectively. Compared to CL-D, serum GPx concentration in crossbred Holstein cows were positively influenced by herbal diets, time ($P<0.001$), and $D \times T$ ($P<0.05$). PT-D, PL-D and LG-D increased the serum glutathione peroxidase (GPx) concentration in Holstein crossbred cows by 9.5, 8.4 and 5.9% and no variation was found between PT-D and PL-D groups. The serum catalase level in Holstein crossbred cows were solely positively influenced by herbal diets ($P<0.001$), and no effects of time and $D \times T$ were found ($P>0.05$). Compared to CL-D, higher catalase concentration was found at PT-D, LG-D and PL-D, respectively. The serum calcium level in Holstein crossbred cows were significantly influenced by time ($P<0.001$), herbal diets ($P<0.05$) and $D \times T$ was not affected ($P>0.05$). The Supplementation of PT-D with CL-D increased the serum calcium concentration. Herbal diets and $D \times T$ exert positive influence on serum P, whereas the serum zinc level was influenced by solely herbal diets. Compared to CL-D, 11.3, 8.9 and 5.5% higher serum zinc concentration in Holstein crossbred cows were obtained at LG-D, PL-D and PT-D, respectively.

Table 56. Serum lipid, antioxidants and mineral profile (mean $\pm$ SE) of dairy cows offered herbal supplementation

Parameters	Diets				SEM	P-value or Effects		
	CL-D	PT-D	LG-D	PL-D		D	T	D × T
Serum lipid profile (mg/dl)								
TG	24.63±0.39	22.61±0.42	20.34±0.40	21.84±0.40	0.185	<0.001	0.172	0.939
TC	171.60±1.92	118.22±2.04	134.44±1.95	131.76±1.94	0.907	<0.001	<0.001	0.001
HDL-C	66.84±0.36	71.26±0.39	67.49±0.37	67.20±0.37	0.173	<0.001	<0.001	<0.001
LDL-C	99.83±1.88	42.44±2.00	62.88±1.91	60.20±1.90	0.889	<0.001	0.002	<0.001
VLDL-C	4.93±0.08	4.52±0.08	4.07±0.08	4.37±0.08	0.037	<0.001	0.172	0.939
Serum antioxidants status								
TAC (mg/L)	191.19±0.68	199.76±0.72	200.23±0.69	201.48±0.68	0.320	<0.001	<0.001	0.027
SOD (% inhibition)	97.39±0.62	106.03± 0.63	104.58±0.63	105.92±0.66	0.292	<0.001	0.016	0.860
GPx (nmol/min/ml)	217.41±1.24	238.02±1.32	230.34±1.26	235.63±1.26	0.588	<0.001	<0.001	0.019
Catalase (nmol/min/ml)	6.67±0.23	8.38±0.24	8.24±0.23	7.55±0.23	0.107	<0.001	0.099	0.940
Serum mineral profile								
Ca (mg/dl)	8.08±0.06	8.29±0.07	8.19±0.06	8.24±0.06	0.028	0.126	<0.001	0.063
P (mg/dl)	4.16±0.04	4.30±0.04	4.19±0.04	4.24±0.04	0.018	0.165	0.001	0.002
Zn (µg/dl)	99.49±0.93	101.19±0.99	104.46± 0.95	102.80±0.95	0.236	0.001	0.493	0.877
Serum immune status (mg/dl)								
TP	6.65±0.06	6.78±0.06	6.68±0.06	6.70±0.06	0.028	0.520	<0.001	0.054
Urea	29.29±0.74	24.79±0.79	27.21±0.75	24.96±0.75	0.349	<0.001	<0.001	0.012
BUN	13.77±0.35	11.65±0.37	12.79±0.35	11.73±0.35	0.164	<0.001	<0.001	0.012
Albumin	3.35±0.07	3.83±0.12	3.67±0.07	3.55±0.07	0.032	<0.001	0.033	0.007
Globulin	3.40±0.11	2.95±0.07	3.01±0.12	3.15±0.12	0.054	0.001	0.002	0.029
Al : Gb	1.00±0.06	1.30±0.07	1.29±0.07	1.18±0.07	0.030	<0.001	<0.001	0.024
IgG	15.28±0.37	19.91±0.37	22.35±0.37	21.97±0.39	0.173	<0.001	0.010	0.886
IgM	2.52±0.11	2.90±0.12	2.78±0.11	2.81±0.12	0.053	0.147	0.233	0.447
Liver health status (U/L)								
AST	40.55±1.25	33.76±1.20	31.38±1.22	32.55±1.19	0.557	<0.001	<0.001	0.039

Parameters	Diets				SEM	P-value or Effects		
	CL-D	PT-D	LG-D	PL-D		D	T	D × T
ALT	17.58±0.57	7.83±0.55	7.92±0.56	6.83±0.54	0.255	<0.001	0.668	0.001
ALP	85.70±0.97	53.34±0.94	77.56±0.95	73.57±0.93	0.434	<0.001	<0.001	0.004

CL-D: German grass, broken rice, wheat bran, mustard oil cake, DCP, and salt; PT-D: CL-D + 100 g plantain powder/day/cow, LG-D: CL-D + 100g lemongrass powder/day/cow; PL-D: CL-D + 50 g plantain and 50 g lemon grass powder, TG: triglycerides, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, VLDL-C: very low density lipoprotein cholesterol, TAC: total antioxidants capacity, SOD: superoxide dismutase, GPx: glutathione peroxidase, Ca: calcium, P: phosphorous, Zn: zinc, TP: total protein, BUN: blood urea nitrogen, Al: albumin, Gb: globulin, IgG: immunoglobulin-G, IgM: immunoglobulin-M, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, $p < 0.05$, SEM: standard error of mean, D: Diet, T: Time

Covariates (PN, DIM and BW) appearing in the model are evaluated at the following values: PN = 2.50, DIM = 105.00, BW = 430.3192.

The serum total protein concentration in Holstein crossbred cows were positively influenced by time ($P < 0.001$) but not herbal diets and $D \times T$ ($P > 0.05$). Herbal diets (D), time ($P < 0.001$) and $D \times T$ ($P < 0.05$) exerts positive influence on serum urea and blood urea nitrogen (BUN) level in Holstein crossbred cows. PT-D, LG-D and PL-D showed the tendency of reducing the concentration of serum urea and BUN. The serum albumin and globulin concentration in Holstein crossbred cows were influenced by herbal diets, time and $D \times T$. The serum immunoglobulin-G (IgG) level in Holstein crossbred cows were influenced by herbal supplementation ($P < 0.001$), and time ($P < 0.05$). LG-D, PL-D and PT-D groups increased the serum IgG concentration in Holstein crossbred cows by 46.3, 43.8 and 30.3%, respectively compared to CL-D. The herbal Supplementation with CL-D showed the tendency of improving the level of serum immunoglobulin-M (IgM) level which is not significant. Incorporation of herbal powder ($P < 0.001$), time ($P < 0.001$) and $D \times T$ ($P < 0.05$) positively influenced the liver enzymatic activity. Compared to CL-D, better AST concentration was obtained at LG-D, PL-D and PT-D, while better liver enzyme ALT was observed at PL-D, PT-D and LG-D. Better ALG concentration was obtained 37.76, 9.28 and 14.15% by PT-D, LG-D and PL-D compared to CL-D.

d. Milk antioxidants status and mineral profiles

Milk antioxidants status and mineral profiles are shown in Table 57. The herbal Supplementation (PT-D, LG-D, PL-D) with control diet (CL-D) significantly increased the level of total antioxidant status in milk compared to CL-D ($P < 0.05$). The antioxidants level in milk (milk DPTH) were influenced by herbal supplementation diet (D), time (T) and diet and time interaction ($D \times T$). Compared to CL-D, milk DPTH are obtained higher at PT-D, PL-D and LG-D, respectively. The milk total antioxidants capacity (TAC) from dairy cows fed herbal supplementation were significantly increased compared to CL-D ($P < 0.05$). The time period and interaction between time and diets ($D \times T$) were affected in terms of milk TAC ($P < 0.05$). Compared to CL-D, about 14.0, 8.5 and 10.5% higher milk TAC were obtained at PT-D, LG-D and PL-D. Antioxidants enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase level in milk were influenced by herbal supplementation diets (D), time (T) and $D \times T$. Feeding solely plantain, or lemon and plantain and lemon mix powder increased the milk SOD level compared to control diet and highest level was obtained at PT-D and then PL-D and LG-D group, respectively. No statistical variation was observed between PL-D and LG-D. Compared to CL-D, milk GPx level was obtained higher at PL-D, PT-D and LG-D by 11.3, 10.8 and 9.92%, respectively. Incorporation of herbal Supplementation with CL-D significantly improved the milk catalase level compared to control ($P < 0.05$). Milk catalase activity was

obtained higher at LG-D, PL-d and PT-D, respectively but no statistical difference was found among the diets.

Table 57. Milk antioxidants and mineral profile (mean± SE) of dairy cows offered herbal supplementation

Parameters	Diets				SEM	P-value or Effects		
	CL-D	PT-D	LG-D	PL-D		D	T	D × T
Milk antioxidants status								
DPPH (%)	19.84±0.25	21.84±0.25	22.26±0.26	22.09±0.25	0.116	<0.001	0.871	1.000
TAC (mg/L)	32.55±0.31	35.73±0.32	37.13±0.33	36.30±0.32	0.149	0.001	0.002	0.693
SOD (%inhibition)	18.10±0.25	22.98±0.26	21.01±0.25	21.71±0.25	0.116	<0.001	<0.001	0.016
GPx (nmol/min/ml)	29.82±0.17	33.05±0.18	33.10±0.17	33.62±0.17	0.080	0.002	0.095	0.633
Catalase(nmol/min/ml)	2.01±0.01	2.10±0.01	2.12±0.01	2.11±0.01	0.004	<0.001	<0.001	0.158
Milk minerals status (mg/kg)								
Ca	1.45±0.01	1.48±0.01	1.47±0.01	1.48±0.01	0.004	0.167	0.027	0.137
P	1.03±0.01	1.07±0.01	1.03±0.01	1.06±0.01	0.003	0.001	0.028	0.249
Zn	3.82±0.02	3.84±0.02	4.89±0.01	3.87±0.01	0.004	<0.001	0.019	0.034
β-carotene	0.26±0.01	0.28±0.01	0.34±0.01	0.31±0.01	0.004	<0.001	<0.001	0.171

CL-D: German grass, broken rice, wheat bran, mustard oil cake, DCP, and salt; PT-D: CL-D + 100 g plantain powder/day/cow, LG-D: CL-D+100g lemongrass powder/day/cow; PL-D: CL-D + 50 g plantain and 50 g lemon grass powder, DPPH : 2,2 -diphenyl-1-picryl hydroxyl; TAC: total antioxidants capacity, SOD: superoxide dismutase, GPx: glutathione peroxidase, Ca: calcium, P: phosphorous, Zn: zinc, Values within rows with different superscript letters are significantly different , $p < 0.05$, SEM: standard error of mean, D: Diet, T : Time

Covariates (PN, DIM and BW) appearing in the model are evaluated at the following values: PN = 2.50, DIM = 105.00, BW = 430.3192.

Feeding solely plantain (PT-D) or lemon (LG-D) and plantain-lemon mix powder (PL-D) with control diet (CL-D) and time period significantly improved the milk calcium level compared to CL-D ($P < 0.05$). PT-D increased the milk calcium level compared to other groups. Milk phosphorous level in Holstein crossbred dairy cows were influenced by herbal diets (D) and time (T). Incorporation of herbal powder with CL-D significantly improved the milk zinc level in Holstein crossbred cows. Compared to CL-D, approximately 5.0, 4.5 and 3.0% higher zinc level in milk were obtained at LG-D, PL-D and PT-D groups, respectively. Milk β-carotene level in Holstein crossbred dairy cows were influenced by herbal powder supplementation (D), time (T) ($P < 0.001$) and not by D × T ($P > 0.05$). Greater amount of β-carotene in milk from Holstein crossbred cows were obtained at LG-D, PL-D, PT-D and CL-D, respectively.

11.15. Mouse model trial

a. Performance

Better live weight gain (LWG) was obtained at fresh plantain extract P5 and acteoside standard group (A5) compared to control (C) group (Table 58). The higher the dose of fresh plantain extract at 5 and 10 mg/kg BW/day reduce the growth of mouse. Better feed efficiency was obtained at fresh plantain extract P5 and acteoside standard group ($P < 0.01$).

Table 58. Performance of mouse offered acteoside standard and different level of fresh plantain herb extract

Parameters	C	A5	P5	P10	P20	LS
Initial wt. (g/m)	26.97	27.75	27.63	25.47	24.10	0.133
Final wt. (g/m)	34.90	36.20	36.23	31.40	29.47	*
LWG (g/m)	7.93	8.45	8.60	5.93	5.37	**
LWG (g/m/d)	1.13	1.21	1.23	0.85	0.77	**
Feed intake (g/m)	63.46	71.64	62.68	68.38	63.43	**
FCR	8.05	8.49	7.32	11.66	11.84	**

C:Control (Formulated feed); A5: C+Acteoside standard (5 mg/BW/day); P5: C+ Plantain's acteoside (5mg/BW/day); P10: C+ Plantain's acteoside (10 mg/BW/day); P20: C+ Plantain's acteoside (20 mg/BW/day); LWG:Live weight gain; FCR: feed conversion ratio; g:gram; m:mouse; LS:Level of significance; *:P<0.05; **:P<0.01.

b. Liver health

Supplementation of acteoside standard and fresh plantain extract through drinking water effective for liver enzymatic activity (Table 59). AST enzymatic activity was better at P5, A5, A10 and A20 group compared to C, whereas ALT better value was found at P10 group. Better serum ALP level was obtained at supplemented groups compared to control ($P<0.01$).

Table 59. Liver enzymatic status of mouse offered acteoside standard and different level of fresh plantain herb extract

Parameters	C	A5	P5	P10	P20	LS
Glucose (mmol/L)	9.16	9.29	9.44	9.33	9.75	*
AST (U/L)	259.96	111.94	102.97	163.20	215.44	**
ALT (U/L)	68.94	46.70	34.06	27.20	46.12	*
ALP (U/L)	69.17	50.17	48.96	41.15	43.05	**
AST:ALT	3.78	2.43	3.01	6.30	5.43	**

C:Control (Formulated feed); A5: C+Acteoside standard (5 mg/BW/day); P5: C+ Plantain's acteoside (5mg/BW/day); P10: C+ Plantain's acteoside (10 mg/BW/day); P20: C+ Plantain's acteoside (20 mg/BW/day); LS:Level of significance; *:P<0.05; **:P<0.01

c. Serum antioxidants

Supplementation of synthetic acteoside standard and fresh plantain extract through drinking water improved the serum antioxidants level (Table 60). Improved SOD level was obtained at A5, P5 and P10 groups respectively, whereas lower value was obtained at C and P20 groups. Synthetic acteoside and herbal acteoside improved the serum GPx level and better value was obtained at P10, P5 and A5 groups respectively ($P<0.01$). Better catalase enzymes activity was obtained at A5 groups, then P10 and P5 groups, respectively.

Table 60. Serum antioxidant status of mouse offered acteoside standard and different level of fresh plantain herb extract

Parameters	C	A5	P5	P10	P20	LS
SOD (U/ml)	91.62	105.89	107.82	107.37	97.30	**
GPx (U/ml)	111.01	117.34	117.66	120.98	108.66	**
Catalase (U/ml)	485.27	526.55	511.56	516.33	493.99	*

C:Control (Formulated feed); A5: C+Acteoside standard (5 mg/BW/day); P5: C+ Plantain's acteoside (5mg/BW/day); P10: C+ Plantain's acteoside (10 mg/BW/day); P20: C+ Plantain's acteoside (20 mg/BW/day); LS:Level of significance; *:P<0.05; **:P<0.01.

11.16. Light microscopic examination

a. Liver

The liver has a unique characteristic morphologic organization, cell composition, and functions. Histological features were found normal in control and all plantain supplemented (5, 10 and 20 mg/kg BW) mice without any abnormalities. Briefly, the parenchymal hepatocytes were located around the central veins or between the portal canals (Fig 22A). Hepatocytes are arranged into hepatic laminae/plates diverging from the central vein and separated by vascular channels (sinusoids) (Fig 22A). Sinusoids are lined by endothelial cells and macrophages (Kupffer cells) (Fig 22 B-C). Expanded areas of interlobular connective tissue among the hepatic lobules containing a branch of the portal vein, hepatic artery, bile duct, and lymph vessel are considered portal canal (Fig 22B). The connective tissue is surrounding the vessels and ducts of the portal canal is called Glisson's sheath (Fig 22B). The hypotheses is that the antioxidant properties of plantain may influence the hepatic macrophages or other immune cell population in the liver.

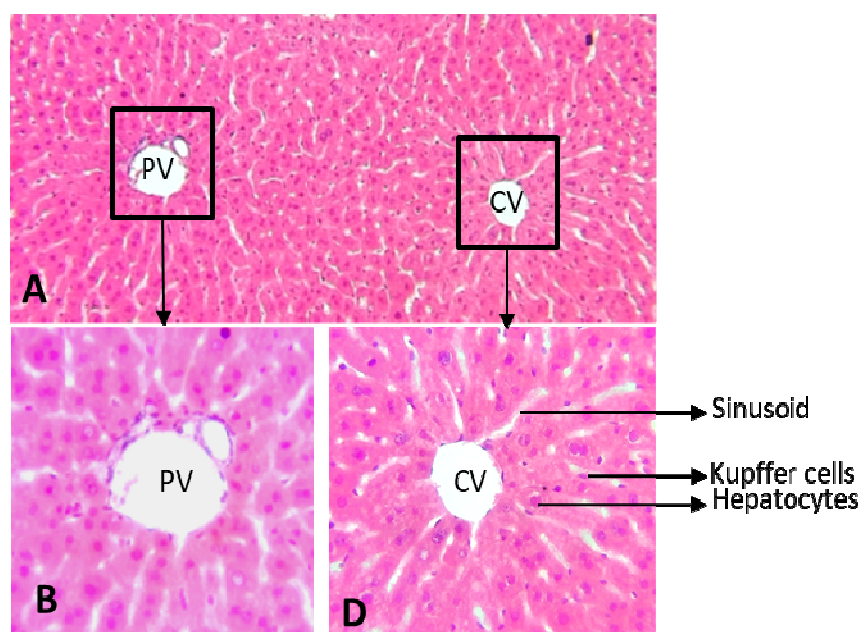


Fig 22(A-D). Tissue sections of plantain submitted (5 mg/kg) mice livers. (A). Showing liver parenchyma containing central vein (CV) and expanded areas of interlobular connective tissue containing portal vein (PV). B. Showing parenchymal periportal areas around the portal canal. The portal canal contains a large portal vein (PV), hepatic artery, bile duct, and lymph vessel). C. Higher magnification showing radiating one cell thick hepatic laminae or plates from the CV with sinusoid and endothelial cells and Kupffer cells.

b. Lymphoid organs

Major lymphoid organs of mice comprise of lymph node, spleen, and thymus. The histological features were found normal in control and all plantain supplemented (5, 10 and 20 mg/kg BW) mice without any abnormalities. Interestingly, immune cell localization was high in the plantain supplemented mice lymph node than control. Representative micro-photograph was given only 5 mg/kg plantain supplemented mice (Fig 23 A-B). Detail study ongoing.

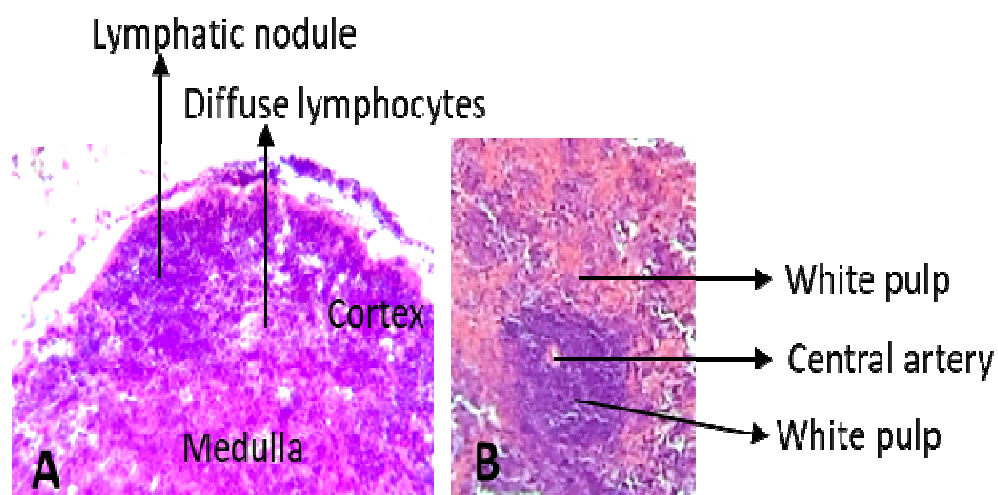


Fig 23(A-B). Tissue sections of plantain supplemented (5 mg/kg) mice lymph node (A) and spleen (A). A. Lymph node consisted of outer cortex and inner medulla. Cortex contain primary lymphatic nodules and diffuse lymphatic tissues. Immune cell localization was high in the plantain supplemented mice lymph node than control. C. Spleen consisted of densely populated lymphocytes areas (white pulp) and scattered lymphocytes containing red pulp. Immune cell localization was high in the plantain supplemented mice lymph node than control mice.

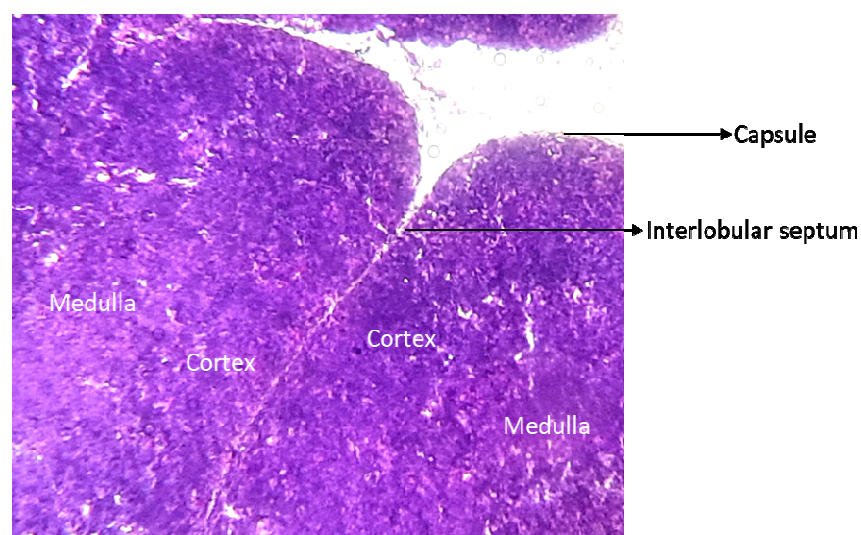


Fig 23 c. Tissue sections of plantain supplemented (5 mg/kg) mice thymus. Thymus consisted of outer cortex and inner medulla. Cortex contain densely populated lymphocytes. Medulla contain comparatively low number of lymphocytes. Immune cell localization was high in the plantain supplemented mice lymph node than control mice.

c. Gastro-intestinal tract Associated Lymphatic Tissues

Mucosa associated lymphatic tissues is important for gut health and immunity. The histological features were found normal in control and all plantain supplemented (5, 10 and 20 mg/kg BW) mice without any abnormalities. Interestingly, villi length and mucosa associated lymphatic tissues (MALTs) were high in the plantain supplemented mice (Fig 24) than control mice. We hypothesises that antioxidant properties of plantain may influence the MALT in the gut mucosa (research ongoing).

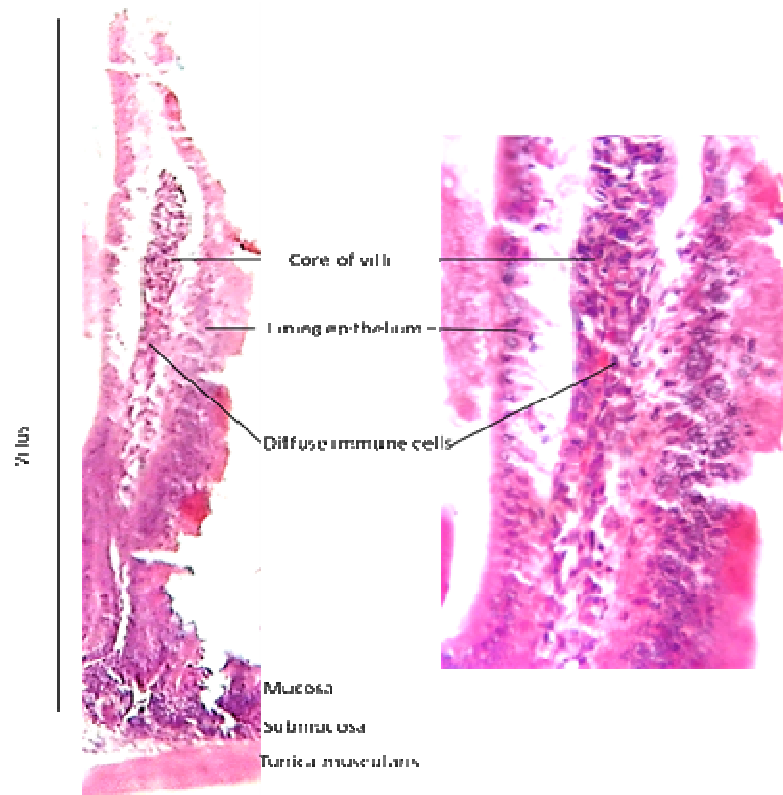


Fig 24. Tissue sections of plantain supplemented (5 mg/kg) mice duodenum. Villi length and mucosa associated lymphatic tissues (MALTs) were high in the plantain supplemented mice. MALTs were high in the plantain supplemented mice than control mice.

12. Research highlights

A. Component-1 (BRR1)

Title of the sub-project: *Fortification and standardization of nutritional level in selected human foods and efficacy test of polyphenolic compounds in livestock products*

Background

Rural to urban migration is one of the major reasons of the exposure of the street children to the vulnerabilities. Considering the increasing trend of street children and diversity of the vulnerabilities, it is very much necessary to take proper and effective steps to protect the children from all types of violations, to ensure their basic necessities properly and to ensure their well growth and development. Although there are several reports, case studies, surveys are available on street children of Bangladesh but very little information are available regarding food formulation especially for their nutritional requirement to address malnutrition. The aim of the component (BRR1) of the sub-project is to intervene at this point to make a concrete step to eradicate malnutrition with formulated rice-based food items. Since rice is an important source of energy, hypoallergenic, easily digested, providing protein with higher nutritional quality and has versatile functional nutraceutical properties, so rice based low cost balanced, nutritious and safe diet formulation will be able to address malnutrition related problems of the vulnerable section of our population especially urban street children.

Objectives

- i. Baseline survey on street children in capital city Dhaka to understand the required calorie gap.
- ii. Formulation of rice-based energy dense bakery food items.
- iii. Impact evaluation study on energy dense rice biscuit (EDRB) food on street children.

Methodology

A semi-structured questionnaire was developed to collect data through face-to-face interview with the respondents. The questionnaires were pre-tested in areas outside our sample and were revised on the basis of feedback received from field-testing. The quantitative questionnaire mainly covered diverse information of study respondents such as socioeconomic and demographic characteristics, current living conditions and livelihood activities, morbidity and health-seeking behavior, dietary intake pattern, and education and drug uses, and anthropometrical measurement such as body weight and height, BMI, waist and hip circumferences ratio, mid-arm circumference. Proximate analysis of energy dense rice-based bakery products was carried out by estimation of fat, protein, fiber, ash, moisture and carbohydrate. Estimation of minerals and heavy metals were evaluated by AOAC (2000) method. A total of 52 respondents including 32 testing and 35 controls samples of both sexes were kept in impact study of formulated food (rice based cake or biscuit) on street children. A free one-hour schooling was set at project field at Uttara near the Turag river side where informal education was given for 4 months. In order to monitor that the serving amount of 100g

equivalent rice cake were properly intake by selected 32 volunteers or respondents at daily basis. Blood samples were taken at day 0 as initial and 100g of serving per day formulated food (rice-based cake or biscuit). On the other-hand controls were not feed the similar amount of 100g of serving per day formulated food (Rice based cake or biscuit) but keeping them on their usual intake of food. Control group for 20 street children were subjected to draw the blood samples as initial too. At the end of 4 months' time blood samples from both samples and controls were taken for comparison of blood parameters specially CBC (Complete Blood Count), Hemoglobin, CRP and Prealbumin. Blood parameters related to malnutrition such as CBC (Complete Blood Count), Hemoglobin, CRP and Prealbumin were collected at both the starting and the end time of the impact survey of selected 52 respondents including testing and control samples.

Key findings

- Baseline survey data on daily calorie intake of street children in capital city, Dhaka reveals 27-59% deficiency of RDI (Recommended Dietary Intake) prevailed in boys population (n=243) and 28-56% gap of RDI for Girls population (n=141).
- Our baseline data revealed that both boys and girls for weight for age and height for age were less than -2σ score which classified as underweight and stunting population of 4-12 yrs and it is also corelated with less than 5th percentile to define shortness and low weight-for-length of our target population for both boys and girls of 4-12 yrs.
- Rice based bakery product can be prepared with nutritionally enriched rice flour. It is low cost, safe and nutritious.
- Rice-based energy dense bakery food products such as EDRB and ERDC have energy density of 5.2 and 5.0 respectively with low moisture content.
- EDRC has positive impact on street children to minimize their malnutritional status by improving their body immunity.

Key words: Energy dense rice biscuit (EDRB), Energy dense rice cake (EDRC), Rice based bakery products. Energy density Street children, Malnutrition.

B. Component-2 (BARI)

Title of the sub-project: *Fortification and standardization of nutritional level in selected human foods and efficacy test of polyphenolic compounds in livestock products*

Background

Fried chips are a kind of mouthwatering food. It is a snacks food that was introduced for the first time in our country in the decade of eighty's. Initially, the market of the product was very small. However, in course of time the size of the market has been expanded significantly. Most of the people like to consume fried chips item commonly potato chips during travelling. Potato chips have been popular salty snacks for 150 years, and retail sales in the USA are worth about \$6 billion/year, representing 33 percent of the total sales of this market (Clark, 2003; Garayo and Moreira, 2002). Chips are fried with deep oil at between 160° and 180°C is characterized by very high drying velocities, which are critical to improve not only the mechanical but also the

structural properties of the potato chips (Baumann and Escher, 1995). The quality criteria of potato chips especially color is very important (Marique *et al.*, 2005) to the consumers, which is critical in the acceptance of the product (Pedreschi *et al.*, 2006).

Acrylamide is formed in potatoes generally in higher frying temperature (150-180°C) which is highly related to the color of potato chips that is suspected to have critical implications for human health because of carcinogenic substance possible existence in the product (Pedreschi and Zuniga, 2009, Pedreschi and Moyano, 2005). Potato chips color is affected by the Maillard reaction resulting of reducing sugars, amino acids, or proteins at the surface. It is also affected by the frying temperature and time (Marquez and Anon, 1986).

Vacuum fryer that is used to prepare chips products from different fruits and vegetables can be an ideal alternative to preparing environmentally friendly, safe and quality fried products. Using this technology, various chips products can be easily processed from various fruits and vegetables such as banana, jackfruit, sweet potato, potato, carrot, pineapple, papaya, taro etc. at the distinct temperature and time. After frying the majority of the oil stays on the surface of the fried products. Therefore, it is very important to use a de-oiling system to remove the excess oil from the surface of the fried product before cooling.

In the above circumstances, the component 2 (BARI) of the sub-project component conducted experiments and developed a simplified BARI low-cost vacuum fryer with de-oiling machine and processing protocol for preparing selected chips products having suitable frying temperature-time combination which will be beneficial for the entrepreneurs and stakeholders especially small scale agro-processors in the country.

Objectives

- i. Baseline survey on chips processing technique, marketing and consumer preference;
- ii. Simplification of vacuum fryer with de-oiling machine and optimization of its operating parameters.
- iii. Technology development on value added products with appropriate packaging of selected agricultural produces (jackfruit, banana, sweet potato); and
- iv. Dissemination of the technologies to stakeholders in selected fruits/vegetables catchment areas.

Methodology

A detailed survey questionnaire having two segments based on hawkers and based on producers were prepared and pre-tested in the selected project areas. A total of fifteen hawkers and five producers were randomly selected. The information on selling, processing, frying technique, frying oil type, frying temperature & time, raw materials, packaging, storage etc. were collected and analyzed during the survey.

A small scale and low cost vacuum frying machine was designed and fabricated in a local workshop. A number of factors were considered in the design of the vacuum frying machine which include the physical, mechanical properties and availability of the spare parts for construction and repairing easily. The designed vacuum fryer was drawn with solidworks 16 software. The vacuum fryer was consisted of three different units of vacuum frying unit, condenser unit and vacuum pump.

The fabricated de-oiling machine basically consists of the de-oiling chamber and control panel. Each unit composed of different spare parts. The de-oiling chamber was comprised of a longitudinal perforated stainless steel made basket. The basket was set with a shaft and enclosed the chamber with plain stainless steel sheet, which was set over the base. To rotate the basket clockwise with high speed the basket shaft connected with a centrifugal motor of which capacity was 1.5 Hp.

Local variety of banana and BARI kola-1 were collected and then thinly sliced, soaked into lemon and salt mixed water solution for 3 hrs. in turmeric mixed solution. Vacuum frying of banana slices was fried using BARI low-cost vacuum fryer at 110-130°C for 8-10 mins and then de-oiled the fried chips at 1400 rpm for 1-2 mins. Foil pack was used for storage of banana chips product. Similarly, BARI SWEET POTATO-7 variety was selected for developing vacuum fried sweet potato chips product. Sweet potato was peeled and sliced at 3-4 mm by hand slicer and then dipped into 500 ppm potassium meta-bisulphite solutions for 10-15 mins. Sliced raw chips were fried at 100-120°C for 10-14 mins and stored in foil pack for 6-8 months. In case of processing of jackfruit chips, matured khaja type jackfruit was selected and sliced. Raw jackfruit slices were frozen at -18°C in deep freeze for 1-2 days. Jackfruit cubes were fried at 100-120°C for 20-25 minutes and de-oiled for 1-2 mins to release excessive oil from the fried chips. Vacuum fried jackfruit chips were packed in HDPE and foil pack for nutritional quality and shelf life study.

Key findings

- Baseline survey reveals that most of the fried chip's processor (more than 60%) are unknown about different hazards, hygienic practices and healthiness.
- Most of the chip's processors changed frying oil during chips frying into deep oil when frying oil turned into cloudy.
- Survey finding revealed that fried potato chips contained higher amount (560 – 10180 µg/kg) of acrylamide which is above the permissible limit as per daily intake for a person's body weight.
- BARI developed small scale low-cost vacuum fryer with de-oiling machine can be used for preparing quality fried chips products and excess oil from fried chips surface can be conveniently released using BARI developed de-oiling machine.
- Suitable frying temperature (100-130°C), frying time (5-25 minutes) and processing protocol were determined for preparation of BARI vacuum fried jackfruit (khaja type, golden flesh, 5 mm thickness), banana (slices, 2-4 mm thickness) and sweet potato (100-120°C, 10-12 mins.), chips products.
- Suitable spices combination (40% salt, 46% sugar, 8% garlic & 6% chili powder) should be used with different vacuum fried chips products.
- Foil pack with nitrogen flushing is suitable for longer storage with keeping quality of vacuum fried chips products.

Key words: Vacuum fried chips, Frying techniques, Chips de-oiling, Foil packing and marketing.



Plate 23. Existing potato chips processing in Joypurhat and Bogura districts.



MoA. Indian Officials



Senior Secretary, MoA. Bangladesh



Ex-DG, BARI with local people



DAM officials with SME

Plate 24. Vacuum frying technology demonstration activities.

C. Component-3 (BAU)

12.1. Research highlights: 01

Title of the sub-project: *Fortification and standardization of nutritional level in selected human foods and efficacy test of polyphenolic compounds in livestock products*

Background

Asia, including Bangladesh, is a treasure trove of medicinal herbs that have been used extensively as human tonics since the dawn of human civilization. Additionally, the increased human health concern about animal-derived products has provoked scientists to shift their focus away from biotechnical additives and toward medicinal herbs (Fons et al 2008; Camy et al 2019; Redoy et al 2020). Not all medicinal herbs have the same potential as livestock feed additives, and the level of phytochemical components plays a critical role in determining whether the herb is beneficial or detrimental to farm animals (Al-Mamun et al 2007). Additionally, not all phytochemicals are equally important as feed additives, and the majority of the available literature indicates that among the various phytochemical components, polyphenol has demonstrated antimicrobial, anti-oxidative, anti-inflammatory, and anti-parasitic activity in both ruminants and poultry (Hausmann et al 2007; Ferrazzano et al 2015; Boamah et al 2016). Thus, prior to applying any novel or available herbs to farm animals, it is necessary to ascertain their phytochemical profile. Among the various phytochemical profiling methods, thin layer chromatography is the least expensive and most reliable for screening purposes, whereas high performance liquid chromatography and gas chromatography are more expensive but essential for detailed profiling. To our knowledge, there is a dearth of published literature on the polyphenolic profile of medicinal herbs available in Bangladesh.

Objective 1

- Profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh

Methodology

Preliminary qualitative phytochemical screening was carried out by thin layer chromatography (TLC) technique according to Shanmugam et al. (2010). In TLC, silica gel was used as the stationary phase and appropriate solvent regarding to phenolotic component type was used as mobile phase. For comparison of extracted residues with routinely used standard phenolics were prepared (Sigma Co.) were prepared by dissolving of 0.1 g of each Powder in 4 ml methanol. On the basis of result of TLC, five herbs were selected for specific phenolic component analysis using high performance liquid chromatography and gas chromatography followed by mass spectrophotometry.

Key Findings

Considering the quantitative polyphenolic profile, and biomass yield, the following herbs were found to have a greater potential as feed additives for farm livestock: plantain (*Plantago lanceolata* L.), ivy gourd (*Coccinia grandis* L.), garlic (*Allium sativum*), mint (*Mentha spp*), and lemongrass (*Cymbopogon citratus*). According to TLC analysis, lemongrass contain all of the selected polyphenolic components, whereas plantain and spear mint are deficient in saponin and glycoside, respectively. Additionally, the level of specific bioactive components in selected herbs along with the variation due to different preservation methods were undertaken, which will aid in determining the dose related response and sustainability of using these herbs in farm animals. It's a novel documentation of wet chemistry of medicinal forage herbs in Bangladesh that will be used by other animal nutritionist for further research.

Key words: Medicinal herbs, Polyphenolic components, Thin layer chromatography, HPLC, GC-MS.

12.2. Research highlights: 02

Title of the sub-project: *Fortification and standardization of nutritional level in selected human foods and efficacy test of polyphenolic compounds in livestock products*

Background

In the past decades, non-nutritive feed additives, steroid, synthetic antioxidants, and growth hormone were applied in animal industries to increase productivity by increasing growth rate (Kamal et al., 2019). At present, the use of feed grade antibiotic growth promoters has declined across the world due to their adverse effects on humans and the environment (Al-Dobaib and Mousa. 2009 and Kamal et al., 2019). Bioactive components of medicinal herbs are capable of modulating metabolic processes and demonstrate positive properties such as inhibition of receptor activities, induction and inhibition of gene expression, immune boost up, increase PUFA in livestock products (Al-Mamun et al., 2007; Al-Mamun et al., 2008; and Redoy et al., 2020). Researcher claimed that bioactive components of medicinal herbs not only improved the quality of meat, milk and egg but also enhanced the shelf life of stored and processed animal sourced products due to their natural antioxidants and antimicrobial properties (Velasco and Williams, 2011). Therefore, present project designed to study the impact of medicinal herbs on sheep, broiler, layer, and dairy cows and set up threshold levels in their diets.

Objective 2

- *In-vivo* trail on poultry, sheep, and dairy cattle to find out performance potentials, correct threshold level, digestion and absorption dynamics and % of PCs retain in the meat, milk and egg.

Methodology

a. Sheep trial : The experiment consisted of a completely randomized design with 32 one-year-old sheep and the basal diet (control) was supplemented with plantain (*Plantago lanceolata* L.) and/or garlic leaf (*Allium sativum*) at the rate of 10g DM/.on growth performance, immunity, rumen histology, serum antioxidants, and meat quality of sheep.

Key findings

Compared with the CL-diet group, the live weight gain and feed conversion ratio were 18–26% and 13–20% higher in herbal-supplemented groups, respectively. Moreover, the herbal-supplemented groups, especially the PG-diet group had higher serum immunoglobulin concentration, antioxidant capacity, and rumen papillae size compared to the control. Besides, the lowest caul fat and pelvic fat levels were observed in the PL-diet group followed by PG-, GL-, and CL-diet groups. In addition, lower mutton ether extract and saturated fatty acid along with higher polyunsaturated fatty acid levels were found in all herbal-supplemented groups. In conclusion, dietary supplementation with plantain and/or garlic leaf might be used as an alternative in sheep to promote growth, health status, and antioxidant rich lean mutton production.

Key words: Plantain, Garlic leaf, Mutton fatty acid, Serum antioxidants, Sheep

b. Broiler trial : The experiment was conducted in a Completely Randomized Design (CRD) containing 3024 Chicks were randomly distributed into 3 equal groups with 3 replicates in open sided shed house each replication having 336 chicks (i.e. $3 \times 3 \times 336 = 3024$ birds). The dietary treatments were T_0 (Formulated diet + AGP (Control)), T_1 (Formulated diet + 1.0 % (DM) Shade dried herb mixture, T_2 (Formulated diet + 1.0 % (DM) Blenching force dried herb mixture. The herb mixture was composed of plantain herb (*Plantago lanceolata* L.), ivy-gourd (*Coccinia grandis*), spearmint (*Mentha spp*), garlic leaves (*Allium sativum*). Routine vaccination and biosecurity were maintained strictly. Feed intake, weekly body weight, mortality, if any, were recorded and on day 32 five birds from each replication were slaughtered to determine carcass characteristics, meat color, shank mineralization, and fatty acid profile of meat.

Key findings

European production efficiency factor (EPEF; involves three factors feed conversion, mortality and daily gain) was improved significantly in T_1 compared to T_0 and T_2 . The herbal groups improved calcium, phosphorus and poly unsaturated fatty acid contents of meat than control and no significant difference was remained between herbal groups. However, T_1 found better compared to T_2 in case of calcium and ω -3 fatty acid accumulation. It was concluded that 1 % shade dried herbs mixture might be recommended for functional broiler production in Bangladesh.

Key words: Broiler, Production performance, Meat quality, Fatty acid profile, Bone mineralization.

c. Layer trial : At 32 weeks, 300 laying hens with similar body weight were randomly allocated to five treatments with five replicates and 12 birds/replication in a completely randomized design until 40 weeks. The maize -soybean based control was supplemented with herbs powder mixture (HPM) at the rate of 0, 5, 10, 15 and 20 g with per kg basal ration. The HPM was prepared by 90% plantain (*Plantago lanceolata* L.), 5% ivy-gourd (*Coccinia grandis*), 3% garlic (*Allium sativum*) and 2% mint (*Mentha spicata*) leaves that were dried under shade at 30-35°C with artificial ventilation system.

Key findings

Supplementation of HPM with basal ration improved egg number, egg weight, egg mass and hen day egg production compared to control group. Egg mass was significantly in HPM supplemented groups compared to HPM0 group. Better egg mass and yolk color were found in HPM15, then HPM20, followed by HPM5, and HPM10 and no statistical variation were observed among HPM supplemented groups. Considering all the parameters, HPM15 is recommended for superior egg production in laying hen.

Key words: Medicinal herbs, Egg production, Egg quality, Laying hen.

d. Dairy trials : Two experiments were conducted (Expt-1 and Expt-2). In expt-1, the animals were fed basal diet with plantain herb powder (PHP) at 0, 50, 100, and 150 g/cow/d, and considering as PHP0, PHP50, PHP100, PHP150, respectively. The basal diet contained German grass, mustard oil cake, wheat bran, commercial feed, broken rice, DCP, and salt that was formulated in accordance with NRC-1985. PHP100 was found best based on milk yield and antioxidant status. Then in expt-2, we used the selected dose in both the plantain and lemon grass or their mixture addition to basal diet, ie Plantain and lemon grass were added at 100g/cow/d and in case of mixture 50g plantain and 50g lemongrass/cow/day. Milk and blood samples were collected on day 14, 28, and 42 of experimental period and analyzed to investigate fatty acid profiles, level of antioxidants.

Key findings

Milk yield, milk fatty acids especially polyunsaturated fatty acids and ω -3 fatty acid contents, level of antioxidants were accelerated through medicinal herbs, plantain, and lemongrass supplementation. Between herbs, plantain was found better considering all milk parameters studied.

Key words: Medicinal herbs, bioactive components, milk antioxidants, dairy cow.

12.3. Research highlights: 03

Title of the sub-project: *Fortification and standardization of nutritional level in selected human foods and efficacy test of polyphenolic compounds in livestock products*

Background

Medicinal herbs have higher capability to scavenge the free radicals (Al-Mamun et al 2007) that might reduce the oxidation leading to raise the milk, meat and egg quality. Moreover, medicinal forage herbs are rich source of flavonoids, glycosides, phenols, alkaloids, terpenoids and tannins that showed the antioxidants properties (Ashwini et al 2012), anti-inflammatory activity (Deshpande et al 2011), and hepato-protective activity (Kumar, A. 2012). Medicinal herbs are aromatic in nature due to its volatile components. So, the available medicinal herbs might be used in safe and quality livestock production including sheep, broiler, layer and dairy cattle. But the threshold level is an important factor as herbs possesses phytochemicals like polyphenols, glycosides, tannins, and saponins which have positive impact on livestock along with human health, but overdose might lead to reduce feed intake, nutrients absorption, bone formation and poisoning not only in livestock (Ebrahim et al. 2015) but also in human (Lorent et al. 2014).

Objective 3

- Validation of specific effect of PCs in both human and animal body through different mouse model.

Methodology

For experimental purpose, the mice were randomly allocated into five groups like; control (C), control with acteoside standard at the 5mg/kg BW/day (A5), control with fresh plantain at the rate of 5 (P5), 10 (P10) and 20 (P20) mg/kg BW/day for a period of 7 days. Each group had 4 mice. Mice were offered standard formulated diet as control. The acteoside standard and fresh plantain extract were supplemented through drinking water. Mice were anaesthetized with formalin during sacrifice on day 7. Blood was directly collected from heart and serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were performed using commercial kits. Histopathology and immunohistochemistry of digestive organs (Stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, spleen) and immune organ bursa tissues were done using standard protocols.

Key findings

AST enzymatic activity was better at P5, A5, A10 and A20 group compared to C, whereas ALT better value was found at P10 group. Better serum ALP level was obtained at supplemented groups compared to control. Improved SOD level was obtained at A5, P5 and P10 groups respectively, whereas lower value was obtained at C and P20 groups. Synthetic acteoside and herbal acteoside improved the serum GPx level and better value was obtained at P10, P5 and A5 groups respectively ($P < 0.01$). Better catalase enzymes activity was obtained at A5 groups, then P10 and P5 groups, respectively. Interestingly, immune cell localization was high in the plantain supplemented mice lymph node than control, and villi length and mucosa associated lymphatic tissues (MALTs) were also higher in the plantain supplemented mice. The increased antioxidant properties and immune response of plantain might be attributed by the hepatic macrophages or other immune cell population in the liver.

Key words: Medicinal herbs, Polyphenolic components, Mouse model, Human health

B. Implementation Status

1. Procurement

Component-1 (BRRI)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment					
i. Portable Hard drive	01	75000.00	01	75000	100% achievement
ii. Desktop with printing accessories and UPS	01	15000.00	01	15000	
(b) Lab & field equipment					
i. Rice polisher	01	165000.00	01	165000.00	100% achievement
ii. Freeze dryer	01	600000.00	01	600000.00	
iii. Vacuum packaging machine	01	270000.00	01	270000.00	
iv. Bakery Oven	01	85000.00	01	85000.00	
v. Bakery dough food mixer	01	65000.00	01	65000.00	
(c) Other capital items					

Component-2 (BARI)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment					
(b) Lab & field equipment					
1. Vacuum fryer (Lab based)	01	1000000	01	997492.50	100% achievement
2. Vacuum fryer (Commercial)	05	750000	05	743933.93	
3. Pigment extractor/extruder	1	450000	1	446786.79	
4. Extruder (Lab scale)	1	250000	1	248786.79	
5. Fruit washing tank	1	50000	1	46786.79	
(c) Other capital items					
1. File cabinet	01	20000	01	20000	100% achievement
2. Shelves	02	30000	02	30000	
3. Bicycle	02	20000	02	20000	

Component-3 (BAU)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (TK.)	Physical (No.)	Financial (TK.)	
(a) Office equipment					
(b) Lab & field equipment					
(c) Other capital items					
i. Book shelf	01	50,000	01	49,700	100% achievement

2. Establishment/renovation facilities

Coordination component: *Not applicable*

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

Component-1 (BRRI): *Not Applicable*

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

Component-2 (BARI): *Not Applicable*

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

Component-3 (BAU)

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
Repair, renovation, and maintenance of Sheep's Crates	1 Package	100%	Not applicable	Not applicable	100% achievement
Laboratory chemicals and apparatus	38 items	100%			

3. Training/study tour/seminar/workshop/conference organized by the sub-project

Description	Number of participants			Duration (Days/weeks/months)	Remarks
	Male	Female	Total		
Inception Workshop (1)	53	6	59	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.)	62+ 63	7+7	139	1+1= = 2 days	
Annual Research Prog. Review Workshop (2 no.)	65+67	8+8	138	1+1 =2 days	
Project Completion Report Review Workshop (1 no)	52	7	59	1 day	
BRRI Workshop (1)	35	15	50	01 day	Internal review workshop
BARI					
Workshop-1	43	07	50	01 day	Internal review workshop
Workshop-2	68	22	90	01 day	Technology dissemination
Training-1	75	55	130	01 day/batch	6 batches
Training-2	85	85	170	01 day/batch	2 batches

C. Financial and Physical Progress (Combined)

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	7112638	7012005	7012005	0	98.59	Not applicable
b. Field research/lab expenses and supplies	10165600	10157196	10157196	0	99.92	
c. Operating expenses	1139405	1118661	1118661	0	98.18	
d. Vehicle hire and fuel, oil & maintenance	937358	937258	937258	0	99.99	
e. Training/workshop/seminar etc.	676400	594400	594400	0	87.88	
f. Publications and printing	739250	627250	627250	0	84.85	
g. Miscellaneous	703563	697979	697979	0	99.21	
h. Capital expenses	3878786	3878486	3878486	0	99.99	
Total	25353000	25023235	25023235	0.00	98.70	

1. Coordination component (BARC)

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1091400	1091400	1091400	0	100.00	Not applicable
b. Field research/lab expenses and supplies	0	0	0	0	0	
c. Operating expenses	108235	108115	108115	0	99.89	
d. Vehicle hire and fuel, oil & maintenance	66946	66946	66946	0	100.00	
e. Training/workshop/seminar etc.	123600	123600	123600	0	100.00	
f. Publications and printing	324500	312500	312500	0	96.30	
g. Miscellaneous	145319	144160	144160	0	99.20	
h. Capital expenses	0	0	0	0	0	
Total	1860000	1846721	1846721	0	99.29	

2. BRRI component

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1995833	1916109	1916109	0	96.00	Not applicable
b. Field research/lab expenses and supplies	2115000	2114600	2114600	0	99.98	
c. Operating expenses	300000	296524	296524	0	98.84	
d. Vehicle hire and fuel, oil & maintenance	210000	210000	210000	0	100.00	
e. Training/workshop/seminar etc.	112000	30000	30000	0	26.78	
f. Publications and printing	100000	0	0	0	0	
g. Miscellaneous	170167	170167	170167	0	100	
h. Capital expenses	1275000	1275000	1275000	0	100	
Total	6278000	6012400	6012400	0	95.77	

3. BARI component

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2558104	2558104	2558104	0	100.00	Not applicable
b. Field research/lab expenses and supplies	1698600	1698600	1698600	0	100.00	
c. Operating expenses	421170	421170	421170	0	100.00	
d. Vehicle hire and fuel, oil & maintenance	460412	460412	460412	0	100.00	
e. Training/workshop/seminar etc.	440800	440800	440800	0	100.00	
f. Publications and printing	264750	264750	264750	0	100.00	
g. Miscellaneous	317378	317378	317378	0	100.00	
h. Capital expenses	2553786	2553786	2553786	0	100.00	
Total	8715000	8715000	8715000	0	100.00	

4. BAU component

In Taka

Items of expenditure/activities	Total approved budget	Fund Received	Actual Expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual Staff Salary	1467301	1446392	1446392	0	98.58	Not applicable
b. Field Research / Lab expenses and supplies	6352000	6343996	6343996	0	99.87	
c. Operating Expenses	310000	292852	292852	0	94.47	
d. Vehicle Hire and Fuel, Oil & Maintenance	200000	199900	199900	0	99.95	
e. Training/ workshop/ seminar etc.	0	0	0	0	#DIV/0!	
f. Publications and printing	50000	50000	50000	0	100.00	
g. Miscellaneous	70699	66274	66274	0	93.74	
h. Capital Expenses	50000	49700	49700	0	99.40	
Total	8500000	8449114	8449114	0	99.40	

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

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
<u>BRRI component</u> Formulation of low cost and nutritious rice-based food items.	Formulation of rice- based bakery products such as Rice cake, biscuit specially EDRB and EDRC.	Rice based bakery products formulation in Bangladesh. Sensory evaluation of these special product.	Rice flour showed immense potential to be utilized in bakery Industries.
<u>BARI component</u> Simplification of selected processing equipment (vacuum fryer) and optimization of its operating parameters	Design of the vacuum fryer with de-oiling machine drawn using solidworks 16 software, machine spare collected and fabricated in local workshop, operating parameters (pump pressure, capacity, heater capacity, centrifugal speed, motor capacity etc.) optimized and machine performance evaluated.	Simplified BARI low cost vacuum fryer with de-oiling machine developed and operating parameter estimated. Machine capacity for raw and processed product figured out.	Quality vacuum fried chips of different agricultural produces prepared using vacuum fryer and vacuum frying machines also multiplied using the specifications in local workshop and help in entrepreneurship

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
			development.
Technology development on value added products with appropriate packaging (fried chips) of selected produce (jackfruit, banana, sweet potato etc.)	Conducted experimentation for development of processing protocol, evaluation of the nutritional quality, sensory attributes and shelf life of vacuum fried chips products, evaluated packaging materials of stored chips products.	Processing protocol of 04 vacuum fried chips products (jackfruit, banana, potato and sweet potato) were developed. Shelf life and nutritional quality of the product were estimated.	Developed processing protocol and other related information will assist to prepare the chips products which will create opportunity to the agro-processors/SME level for marketing the product in domestic and export market.
Dissemination of the technologies to stakeholders (grower association, co-operative society, agro-processors, SME etc.) in selected fruits/vegetables catchment areas	Technology was disseminated to the farmers, traders, agro-processors etc. by consultation, visiting the facility, hands on training, workshop, product testing and demonstration/display.	Technology introduced among the stakeholders and farmers, agro-processors etc. practically trained up.	Trained personnel involved to the technology dissemination and product marketing activities. Economic activities and earning will increase.
BAU component a) Profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh	Selection of herbs, collection of herbal seeds and cloves Land preparation and herbs cultivation Preliminary screening of herbs through thin layer chromatography (TLC) Determination of TPC and TFC of some selected herbs Phytochemical profiling of herbs through HPLC followed by mass spectrophotometry for known herbs.	Through this sub-project we have able to quantify the phytochemical presence or absence in the selected herbs. Then, the threshold level of plantain herb, garlic leaves and ivy-gourd leaves for improved growth and antioxidants enriched, low cholesterol mutton production have been determined. Then a novel	Ensured the consumption of antioxidants enriched milk, meat and egg for very few people related to this sub-project. Finally, the plantain herb technology has been proved as safe and having capability to improve the antioxidants enzymes and liver health for human, that is validated through mouse
b) <i>In-vivo</i> trial on poultry (broiler and layer), sheep and dairy cattle to find out performance potentials, correct threshold level, absorption	An on-station sheep feeding trial was performed using solely plantain or garlic leaves and their mix to assess the growth performance, serum lipid profiles, serum antioxidants status, immune status, meat quality and carcass characteristic. An on-station broiler feeding trial was performed using plantain, spearmint, garlic and ivy-gourd mix powder by		

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
dynamics and % of PCs retain in the meat, milk and egg	observing growth performance, meat nutritional value, and shank mineralization. An on-station sheep feeding trial was performed using plantain and ivy-gourd and their mix to assess the growth performance, meat quality and carcass characteristic. An on farm layer feeding trial was performed using plantain, ivy-gourd, garlic and spearmint mix at 0,5,10, 15 and 20g/kg feed to evaluate the laying hen performance, egg external and internal quality. For the fortification of milk yield and quality, an On-farm dairy cow feeding trial was performed using medicinal forage herb plantain at 0, 50, 100 and 150g/day/animal for the determination of threshold level in dairy cow.	herbal mixture using plantain, spearmint, ivy-gourd and garlic leaves have been developed at optimum threshold level for broiler and layer birds. This outstanding herbal mixture has capability to increase the growth performance, shank minerals contents, and level of antioxidants in broiler. In addition, this technology improved the egg production, egg yolk color and egg quality of commercial laying hen.	model.
c) Validation of specific effect of PCs in human body through mouse model	A validation trial using bioactive components from medicinal herbs and standard on mouse was conducted to assess the liver health, digestive tract and serum lipid and antioxidants level. A total of 15 male mouse aging 5-weeks were randomly allocated into 5 dietary treatments named control, acteoside standard-5 mg/kg BW, acteoside from herbal plants (Plantain) at 5 mg, 10 mg and 20 mg /Kg BW.		

E: Information/Knowledge generated/Policy generated

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Component-1 (BRRI)			
Baseline survey of street children on their daily dietary intake.	Baseline survey on 384 street children in 20 hot spots on Dhaka city.	A baseline survey on street children of Dhaka city was conducted to get the recommended daily intake (RDI) Calorie gap of the population.	The acquainted data will help to prepare suitable nutritional program for the population.
Formulation of low cost and nutritious rice-based food items.	Formulation of rice- based bakery products such as Rice cake, biscuit etc.	Rice based bakery products formulation in Bangladesh	Rice flour showed immense potential to be utilized in bakery Industries.
Impact study of rice-based food on street children	Conducted a four months long impact study on rice product in selected street children.	Nutritional improvement by intaking rice-based products as supplementary diet.	Rice based energy dense items can be utilized in nutritional program and disaster management program in Bangladesh.
Component-2 (BARI)			
Baseline survey conducted on existing status of fried potato chips from processing to marketing channel.	Hawker and producer based survey questionnaire were prepared and pre-tested. Information collected from 160 respondents at 08 selected locations.	Hygienic condition, healthiness, frying technique etc.and nutritional quality (fat, vitamin A & C, acrylamide content) with shelf life of fried chips were evaluated.	Information generated on existing status and practices of local fried chips from processing to marketing.
Simplification of selected processing equipment (vacuum fryer) and optimization of its operating parameters.	Designed and fabricated a small scale vacuum frying machine with de-oiling machine in local manufacturer.	BARI low-cost vacuum fryer and de-oiling machine developed. Capacity of vacuum fryer for raw (700-800 g/batch) and processed chips (350-450 g/batch) product estimated.	Quality vacuum fried chips of different agricultural produces prepared using the machine.
Technology development on value added products with appropriate packaging (Fried chips) of selected produce (jackfruit, banana, sweet potato, carrot etc.)	Conducted experimentation for processing protocol development, packaging effect and quality evaluation of jackfruit, banana, sweet potato, carrot etc. chips products.	Low free fatty acid (>3%), below permissible limit of acrylamide and other nutritional quality were obtained. Suitable frying temperature (110-130°C) and frying time (5-25min.) were also determined.	Stakeholders will be benefitted to prepare the quality vacuum fried chips products.

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Dissemination of the technologies to stakeholders (grower association, co-operative society, agro-processors, SME etc.) in selected fruits/vegetables catchment areas	Dissemination of information to the farmers, traders, agro-processors, end users etc. by consultation, meeting, training, workshop, product testing, demonstration etc.	Increasing awareness creation among the stakeholders for vacuum fried chips products.	Information on vacuum fryig technology will ensure nutritional security of the fried chips products to the stakeholders.
Component-3 (BAU)			
i. Profiling of polyphenolic components (PCs) in available forage herbs in Bangladesh. ii. <i>In-vivo</i> trail on poultry, sheep, and dairy cattle to find out performance potentials, correct threshold level, digestion and absorption dynamics and % of PCs retain in the meat, milk and egg. iii. Validation of specific effect of PCs in both human and animal body through different mouse model..	- Field assistant, contact labor and part time accountant salary. - Herbs, Herbal cloves/seeds purchasing. - Cultivation of plantain and other herbs and their preparation as powder for broiler, layer, and sheep trial. - Broiler trial and its sample analysis. - Land preparation and sowing of selected herbs again for sheep and layer trial. - Feed, layer birds, mouse and sheep were procured and reared. - Layer trial and sheep trial. - Layer trial sample analysis. - Dairy cattle trial and sample analysis. - Chemicals and apparatus, animal feed. - Mouse model and its sample analysis.	Through this sub-project we have abled to quantify the phytochemical presence or absence in the selected herbs. Then, the threshold level of plantain herb, garlic leaves and ivy-gourd leaves for improved growth and antioxidants enriched, low cholesterol mutton production have been determined. Then a novel herbal mixture using plantain, spearmint, ivy-gourd and garlic leaves have been developed at optimum threshold level for broiler and layer birds. This outstanding herbal mixture has capability to increase the growth performance, shank minerals contents, and level of antioxidants in broiler. In addition, this technology improved the egg production, egg yolk color and egg quality of commercial laying hen.	Ensured the consumption of antioxidants enriched milk, meat and egg for very few people related to this sub-project. Finally, the plantain herb technology has been proved as safe and having capability to improve the antioxidants enzymes and liver health for human, that is validated through mouse model.

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

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name etc.)
	Under preparation	Completed & published	
A. Component-1 (BRRI)			
Technology bulletin/ booklet/leaflet/flyer etc.		02	Nova Publication, USA BRRI Publication, Bangladesh
Journal publication	01	03	Acta Scientific Nutritional Health (Under Review Process), India. Bioresearch Communications (BRC), Bangladesh. Journal of Food Nutrition and Health, USA. EC Nutrition, Australia.
Video clip/TV program			
News Paper/Popular Article		01	Nodi Barta of River Foundation
Other publications, if any		4 seminars	Rice congress 2018, Singapore. Bangladesh society of Biochemistry and Molecular Biology, Dhaka University. BCSIR Conference 2019, Dhaka. Annual Conference of Biochemistry Department 2019, BUHS, Dhaka
B. Component-2 (BARI)			
Journal publication	03	01	Baseline survey on existing status of fried potato chips from processing to marketing in selected locations of Bangladesh. Bangladesh Journal of Agricultural Research
Poster printing	01		Scientific Seminar for SAU
Folder printing		03	Processing of vacuum fried jackfruit chips, banana chips and project activities including research findings
Booklet printing	03		BARI low-cost vacuum fryer, BARI de-oiling machine & Application of vacuum frying technology for preparing quality fried chips
Newspaper/popular article	02	05	Kalerkantho, Bonik barta, Prothom alo, The daily Observer, Daily Sun etc.
Technology bulletin (Factsheet printing)	03	03	BARI low-cost vacuum fryer, Processing of vacuum fried jackfruit chips & vacuum fried banana chips (Bangla & English version)
Video clip	01		Vacuum frying technology for value addition of agricultural produces
TV program/News		04	ATN Bangla, ATN News, Channel 24, BTV
Other publications (Patent design)	03		BARI low-cost vacuum fryer, BARI de-oiling machine, BARI vacuum fried jackfruit chips
C. Component-3(BAU)			

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name etc.)
	Under preparation	Completed & published	
Technology bulletin/booklet/leaflet/flyer		1	Title: BAU-Plantive will increase egg production with reduced cost <i>In the Souvenir of BAU Journalist Association</i>
Journal publication	02	02	M.R.A. Redoy, A.A.S. Shuvo, L. Cheng and M. Al-Mamun (2020) . Effect of herbal supplementation on growth, immunity, rumen histology, serum antioxidants and meat quality of sheep. Animal 14(11), 2433- 2441. https://doi.org/10.1017/S1751731120001196 . M.A. Rahman, D. Ray, M.R.A. Redoy and M. Al-Mamun (2021) . Dose titration of herbs mixture powder supplementation on laying performance and egg quality in commercial layer chicken. Livestock Research for Rural Development 33(1). http://www.lrrd.org/lrrd33/1/aliar3313.html . Feasibility analysis of plantain (<i>Plantago lanceolata</i> L.) supplementation on commercial broiler production at the farmer level. Brazilian Journal of Poultry Science (<i>under review</i>). Fortification of milk antioxidants using phytogetic components of natural herbs. Dairy Science (<i>preparing for submission</i>).
Video clip	01		Title: “Green additive to produce functional meat, milk, and egg” https://www.youtube.com/watch?v=N9UbTgrUqLM

G. Description of generated Technology/Knowledge/Policy

i. Technology Factsheet

Component-1 (BRRI)

Technology fact sheet: 01

Title of the technology: *Development of Energy Dense Rice Biscuit (EDRB)*

Introduction

Grain Quality and Nutrition Division (GQND) of Bangladesh Rice Research Institute (BRRI) has developed a low cost, safe and nutritious rice-based bakery product which is suitable for Bangladeshi population specially for children. EDRB is energy dense (ED:5.2) which can generate 515 kcal of energy per 100g of serving. Since EDRB is a dry bakery food item with high nutritional value, it can potentially be used in disaster management program and school feeding nutrition program in Bangladesh to minimizing malnutrition.

Description

We have formulated our rice-based products with several ingredients including rice flour, grinded sagu powder, sugar, skimmed milk, egg, vanilla essence, grinded and extract of aloe vera as lubricating agent, butter, yeast powder, Rice Bran Oil (RBO) and vegetable edible oil. Its moisture content is 3.6%. This product is free of heavy metals such as Arsenic, Lead, Cadmium and Nickel.

Suitable location/ecosystem

All wheat-based bakery industries of Bangladesh can potentially use the rice-based technology.

Benefits

Since rice do not contain any gluten protein, so there is no hypersensitive reaction in human body. This particular product is suitable for children, especially autistic children and old age people. EDRB is energy dense bakery food product and it can generate 515 kcal per 100g serving. EDRB can potentially be used in school feeding nutrition program and disaster management including refugee crisis, flood or cyclone related emergency relief operation program to mitigate malnutrition and emergency response of energy dense dry food as supplements.

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Technology fact sheet: 02

Title of the technology: *Development of Energy Dense Rice Cake (EDRC)*

Introduction

Grain Quality and Nutrition Division (GQND) of Bangladesh Rice Research Institute (BRRI) has developed a low cost, safe and nutritious rice-based bakery product which is suitable for Bangladeshi population specially for children. EDRC is energy dense (ED: 5.0) which can generate 500 kcal of energy per 100g of serving. Since EDRC is a dry bakery food item with high nutritional value, it can potentially be used in disaster management program and school feeding nutrition program in Bangladesh to minimizing malnutrition.

Description

We have formulated our rice-based products with several ingredients including rice flour, grinded sago powder, sugar, skimmed milk, egg, vanilla essence, grinded and extract of aloe vera as lubricating agent, butter, yeast powder, Rice Bran Oil (RBO) and vegetable edible oil. Its moisture content is 5.0 %. This product is free of heavy metals such as Arsenic, Lead, Cadmium and Nickel.

Suitable location/ecosystem

All wheat-based bakery industries of Bangladesh can potentially use the rice-based technology.

Benefits

Since rice do not contain any gluten protein, so there is no hypersensitive reaction in human body. This particular product is suitable for children, especially autistic children and old age people. EDRC is energy dense bakery food product and it can generate 500 kcal per 100g serving. EDRC can potentially be used in school feeding nutrition program and disaster mangement including refugee crisis, flood or cyclone related emergency relief operation program to mitigate malnutrition and emergency response of energy dense dry food as suppliments.

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Component-2 (BARI)

Technology Factsheet: 01

Title of the technology: *Development of Low-cost BARI vacuum frying machine*

Introduction

Frying is a common food processing method where cooking oil is used as a means of heat transport to fry a product that comes in direct contact with the food. Traditionally, using small amount oil or dipping it in whole oil is used to fry a variety of food items which in many cases are not up to standard. But the vacuum frying method can be used to make a variety of healthy and quality chips products that have added a new dimension to food processing. Generally, fruits or vegetables having higher sugar content are browned when fried in oil at a temperature between 150 - 160°C or higher. Research findings has shown that uncontrolled oil temperature produced excessive carcinogenic substance 'acrylamide' in fried chips products, which lead to cancer in the human body. In developed countries, vacuum fryer is used to prepare chips products from different fruits and vegetables commercially, which helps in preparing quality fried chips while reducing the health risks. Vacuum frying is an advanced method where the quality of the fried chips is maintained at lower temperature with pressure. This technology has great potential in the Snacks Processing Industry of Bangladesh. It is an ideal alternative to preparing environmentally friendly, safe and quality fried products. Using this technology, various chips products can be easily processed from various fruits and vegetables such as jackfruit, pineapple, potato, banana, sweet potato, carrot, papaya, taro etc. at the distinct temperature (100 - 130°C) and frying time (5-25 minutes). The low-cost vacuum frying (VF) machine developed by BARI will undoubtedly assist SME level in the agro-processing industry to play an important role in increasing family income and alleviating poverty by producing various types of fried chips products.



Vacuum fryer & fried chips

Main parts of vacuum frying machine

Vacuum frying machine basically consists of two basic parts/units:

a) vacuum frying unit; b) de-oiling unit

Vacuum frying unit

- Vacuum pump (1 hp)
- Pressure meter (Capacity: -60 mm/Hg)
- Heater (4000 watts, 220 volts, AC)
- Sample holder (800-1000 g)
- Temperature controller unit (Temperature probe/Thermostat)
- Frying chamber (Capacity: 15-20 liters)
- Condenser unit (1-2 liters)

De-oiling unit

- Sample holder (800-1000 g)
- Electric motor (1.5 hp)

Description of vacuum frying process

Flat with dry area will be selected for the device and then will be connected with the electrical line to the control panel line.

The vacuum pump needs to be filled with 1-2 liters condenser oil.

The frying chamber should be filled with 15-20 liters of vegetable oil/other oil and then the heater should be switched on and the frying chamber should be ventilated or fully vacuumed by turning on the vacuum pump.

It needs to specify the temperature (100-130°C) of the oil for frying the chips (5-25 minutes) product in the control panel thermostat system.

The raw sliced chips (2-5 mm) should be placed in the sample holder of the frying unit after rising the cooking oil temperature (100-130°C) and then will be covered by the lid.

It is required to open the steam outlet valve and start the vacuum pump so that the air is fully vacuum.

The water line should be connected in the condenser unit and the inlet valve should be reopened by turning off the vacuum pump when the chips are fried for a certain time.

The lid should be opened from the frying unit and will be take out the fried chips with the basket and then will be de-oiled for few minutes so that the surface oil comes out from the chips.

Fried chips will be packed in the foil pack after de-oiling and then flushed with nitrogen, sealed the packet well.

Suitable area/location

This machine will be applicable all over the country to produce quality vacuum fried chips products using different types of fruits and vegetables.

Benefits

- The price of the device is much cheaper than the imported device.
- The machine is small in size and can be easily moved anywhere and placed in a small space.
- The device is very easy to operate so anyone can use it easily.
- The device can be fabricated in a local workshop and repaired and maintained at low-cost.
- Quality fried chips (jackfruit, pineapple, potato, banana etc.) canbe easily prepared from different produces.

Precautions

- The machine needs to be cleaned immediately after use.
- Water and electrical lines should be properly connected and monitored before starting the machine.

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Technology Factsheet: 02

Title of the technology: *BARI vacuum fried banana chips processing*

Introduction

Banana is a popular and nutritious fruit in the world. It gives energy to the body. The fruit is rich in sugars, vitamins A, B, C, calcium and iron. It is cheaper than other fruits and are available almost all year round. Lack of improved processing and handling technology leads to quantitative and qualitative loss of banana fruits. Processing of banana into fried chips is a simple preparing technique through which a significant amount of postharvest loss of banana should be minimized and the entrepreneur can earn money by preparing fried banana chips product. Vacuum frying (VF) method has incorporated a new dimension in the Snacks Processing Industry to provide healthy and safe fried chips. Generally, fruits/vegetables having higher sugar content are browned when chips fried into oil at a temperature between 150-160 °C or higher. Research findings has shown that uncontrolled oil temperatures produce excessive carcinogenic substance ‘acrylamide’ in fried chips products, which lead to cancer in the human body. In developed countries, vacuum fryer is used to prepare chips products from different fruits and vegetables commercially, which helps in preparing quality fried chips while reducing the health risks. VF is an advanced method where the quality of the fried chips is maintained at lower temperature with pressure. Quality banana chips should be prepared to create vacuum at certain temperature and pressure by controlling temperature of the cooking oil. Although this technology is new in our country but it is widely used in producing fried chips from various fruits and vegetables commercially.

Required materials and equipment

- BARI Kola-1/Meher Sagar/Nepali Sagar raw banana
- Boti or stainless steel (SS) knife
- Hand slicer
- Measuring jars/containers
- Net/strainer/chalni
- Large bowl/saucepan
- Lemon juice /citric acid
- Soybean oil
- Sugar, bit salt and mixed spices (turmeric powder, garlic powder, chili powder etc.)

Description of processing method

- Matured and uniform size of raw banana of BARI Khola-1/Meher sagar/Nepali sagar will be selected and then peeled with a slicer or SS knife.
- Banana will be peeled and then soaked into citric acid (1 gm/L water) or lemon juice (1/4 part of 1 pc lemon/L water) mixed water.
- Raw slices should be prepared as round shape with a hand slicer (3-4 mm thick) and will be soaked into 5% turmeric powder solution for 30 minutes. Peeled banana should also be sliced longitudinally.
- Banana slices will be kept in a net/strainer to remove water from the surface of the raw chips.
- The vacuum pump will be switched on and should be kept raw chips (500-600 g/batch) in the frying chamber and then covered the lid and will be fried for 8-10 minutes.
- The oil temperature should be set at thermostat system between 110-130°C before frying starts.
- In order to separate the excess oil from fried chips product, immediately the chips with basket will be placed in the de-oiling machine for 1-2 minutes.
- Fried banana chips should be packed in polypropylene packet or foil pack and then flushed with nitrogen gas and sealed the packet well which will be used commercially.



Steps for Preparing Banana Chips

Suitable area/location

This technology will be applicable all over the country especially banana growing areas to produce quality vacuum fried banana chips products.

Benefits

Quality fried banana chips should be easily prepared using BARI developed vacuum fryer. It will add value to green banana through processing and will reduce post-harvest loss of banana fruits.

The technology will create new entrepreneurs by increasing family employment.

Precautions

- It is required to select the appropriate maturity of green banana into fried chips products.
- Raw sliced chips will be fried properly according to the set temperature with frying time.
- Fried banana chips should be packed in appropriate packet and sealed properly.

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Technology Factsheet: 03

Title of the technology: *BARI vacuum fried jackfruit chips processing*

Introduction

Jackfruit is a healthy and nutritious seasonal fruit and it is recognized as the national fruit in Bangladesh. It contains various nutrients including thiamine, riboflavin, calcium, potassium, iron etc. Generally two types of jackfruits are available in our country such as Khwaja and Gala. Khwaja type jackfruit bulbs are large in size, less juicy after ripening and relatively hard or crunchy. The bulbs of ripe jackfruit are filled with juice but strong smell so that attractive chips can be prepared by processing the matured jackfruit. Processing of jackfruit into fried chips is a simple processing technique through which a significant amount of postharvest loss of jackfruit should be minimized and the entrepreneur can earn money by preparing jackfruit chips.

Chips are traditionally prepared into dipping oil and frying in an open frying pan, with a variety of fruits and vegetables that are not standardized in many cases. The vacuum frying method should be utilized to prepare quality fried chips that can add a new dimension to the Snacks Processing Industry to provide healthy and safe fried products. Generally, fruits/vegetables having higher sugar content are browned when chips fried into oil at a temperature between 150-160°C or higher. It is observed in research findings that uncontrolled oil temperatures produce excessive carcinogenic substance 'acrylamide' in fried chips products which lead to cancer in the human body.



Steps of Jackfruit Chips Processing

Therefore, many countries around the world use vacuum frying technique to prepare chips from fruits/vegetables commercially that reduce the health risks. Quality jackfruit chips should be prepared to maintain lower temperature with pressure. Although the technology is new but it has potential to prepare fried jackfruit chips commercially in the country.

Required materials and equipment

- Matured Khwaja type jackfruit (not fully ripe, yellowish bulb)
- Boti/Stainless steel (SS) knives
- Peeling machine/Hand slicer
- Chopping board
- Large bowl/saucepan
- Vacuum frying and de-oiling machine
- Soybean oil
- High-density polyethylene (HDPE)/polypropylene packet (PP)/foil pack
- Nitrogen flushing system/Sealing machine etc.

Description of processing method

- Matured with uniform size of khwaja type jackfruit should be selected and then cut it in half with a sharp knife.
- The outer hard cover/upper skin and hard core in the middle of the fruit will be removed by peeling machine/SS knife.
- The bulbs of the jackfruit need to be gently separated or removed. Jackfruit seeds should be separated carefully to avoid bulb disruption.
- The upper and lower part of the bulb will be cut at uniform size and then sliced the bulb vertically size at 5 mm thickness using the SS knife/slicer.

- Raw slices will be packed in HDPE packet and stored in deep freezer at -18°C for 1-2 days. Jackfruit slices stored in the deep freezer will be suitable for frying directly in a vacuum frying machine.
- The frying chamber will be filled with 10-15 liters of soybean oil/mustard oil and heated until the oil temperature rise between 110-120°C.
- Jackfruit slices will be dipped into oil and fried for 20-25 minutes in frying chamber. After frying completed, jackfruit chips with basket will be immediately transferred to de-oiling machine and the machine will be run for 2-3 minutes.
- Fried chips should be packed in PP/HDPE/Foil pack and then flushed with nitrogen gas and sealed the packet well that will be used commercially.
- Jackfruit chips can be easily stored at ambient temperature for 2-3 months in nitrogen flush HDPE packet and more than 6-8 months in foil packet.

Suitable area/location

This technology will be applicable all over the country especially jackfruit growing areas to produce quality vacuum fried jackfruit chips products.

Benefits

- Quality fried jackfruit chips should be easily prepared using BARI developed vacuum fryer.
- It will add value to jackfruit through processing and will reduce postharvest loss of jackfruit.
- The technology will create new entrepreneurs through commercialization.

Precautions

Matured khwaja type jackfruit must select for preparing vacuum fried jackfruit chips.
Raw slices will be fried properly according to the set temperature with frying time.

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Component-3 (BAU)

Technology Factsheet: 01

Title of the technology: *Production of antioxidant rich mutton production by the application of medicinal herbs*

Introduction

The beneficial effects of herbs on farm livestock are observed through the improvement of feed intake, nutrient digestibility, immune system modulation, endocrine system stimulation and intermediate nutrient metabolism (Abou-Elkhair et al., 2014). Over the previous decade, use of herbs or botanicals to boost productivity in livestock has been examined by researchers (Al-Mamun et al., 2007; Cheng et al., 2017). Many herbs or botanicals are rich sources of antioxidants that act as efficient agents for improving the production performance of sheep in tropical and subtropical regions. Due to higher temperature and humidity levels in these regions, sheep organism produces more free radicals that are one of the main constraints on productivity. The presence of bioactive components, such as acteoside, aucubin and catalpol in plantain (PL; *Plantago lanceolata* L.), leads to increase superoxide (free radical) scavenging activities (Al-Mamun et al., 2007). It also possesses laxative effects and enzyme inhibitory activities and improves rumen fermentation in farm animals (Al-Mamun et al., 2007). Moreover, garlic leaf (GL; *Allium sativum*) contains significant levels of flavonoid and polyphenolic components (Chung, 2006), and these bioactive components scavenge free radicals within the animal. At the same time, it contains several sulphur compounds (Martins et al., 2016) that modify rumen microbial fermentation and improve sheep performance (Panthee et al., 2017). Previous research illustrated that 1.0 to 1.3 g DM of PL and GL supplementation per kg of live weight of sheep fed with total mixed ration (TMR) pellets or mixed hay improved their performance (Panthee et al., 2017; Shuvo et al., 2017). Despite these advantages, there is a dearth of information available regarding the efficacy of supplementing PL or GL alone or combined on the serum immunity, serum antioxidants and meat fatty acid profile of sheep. Thus, we hypothesized that the supplementation of PL or GL alone or together might positively affect sheep productivity and meat quality. Therefore, the objectives of the present study were to determine the effect of herbal supplements on growth performance, nutrient digestibility, plasma metabolites, enzyme activities, rumen histology, serum immunity, serum antioxidants, carcass characteristics and meat fatty acid profile of sheep.

Description

Medicinal herb, plantain could be cultivated during November to April. Plantain was found grown well during the atmospheric temperature ranges from 6-24 °C and rainfall ranges from 90-240 mm (Al-Mamun *et al.* 2008). At 60 days of age first cutting of plantain is performed in late December to early January, then 2nd cutting is in late February to early March and final cutting is in late April to early May. After cutting the leaves need to be shade dried with forced air flow at room temperature, then grinding to mesh and finally added to pellet. Garlic is cultivated during winter and after harvesting the residues (leaves along with stem) is procured and shade dried at room temperature through forced air flow, grind to mesh and finally added to pellet.

Individually, plantain and garlic is added at the rate of 10 g DM/sheep/day while in case of mixture of plantain and garlic they are added at the rate of 5 g DM/sheep/day.

Suitable location/ecosystem

We procured sheep from Jamuna river basin, coastal region and barind areas of Bangladesh. Therefore, it is suggested that this technology might be used throughout the country for producing low fat, antioxidant rich mutton production.

Benefits

Many consumers regard red meat as a health hazard because of the low level of beneficial polyunsaturated fatty acids and the predominance of saturated fatty acids. Dietary inclusion of plantain (*Plantago lanceolata* L.) and/or garlic leaf (*Allium sativum*) into a total mixed pelleted ration in sheep substantially improved the level of mutton polyunsaturated fatty acids. Besides, these supplements improved immunity and serum antioxidant levels, which triggered better nutrient utilization, growth performance and health status of sheep. So sheep producers are invited to use these herbal supplements with total mixed pelleted ration for obtaining healthy sheep and value-added mutton production. This herbal supplement is safe for human body with health benefits that was validated through mouse model.

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Technology Factsheet: 02

Title of the technology: *Development of BAU-MIX: A green additive for safe and functional broiler production*

Introduction

Antibiotic growth promoters (AGPs) have been widely used in animal industry for decades. In recent years, the use of antibiotic in animal feeds is facing reduced social acceptance due to the increasing public concern about the bad residual effects of antibiotics known as antimicrobial resistance (AMR) or Superbug on human health. Plantain (*Plantago lanceolata* L.) is important from the animal feeding point of view because of its medicinal value for animals, due to presence of bioactive components like acteoside, catalpol and aucubin it has higher superoxide anion radical scavenging activities that helps to improve the anti-oxidative activity of animals, hence leading to improved animal health (Al-mamun et al., 2007). So, it can be used as alternative to commercial antioxidant for better growth, feed intake, feed conversion ratio, performance index and carcass characteristics of broilers (Chacrabati et al., 2013), and sheep

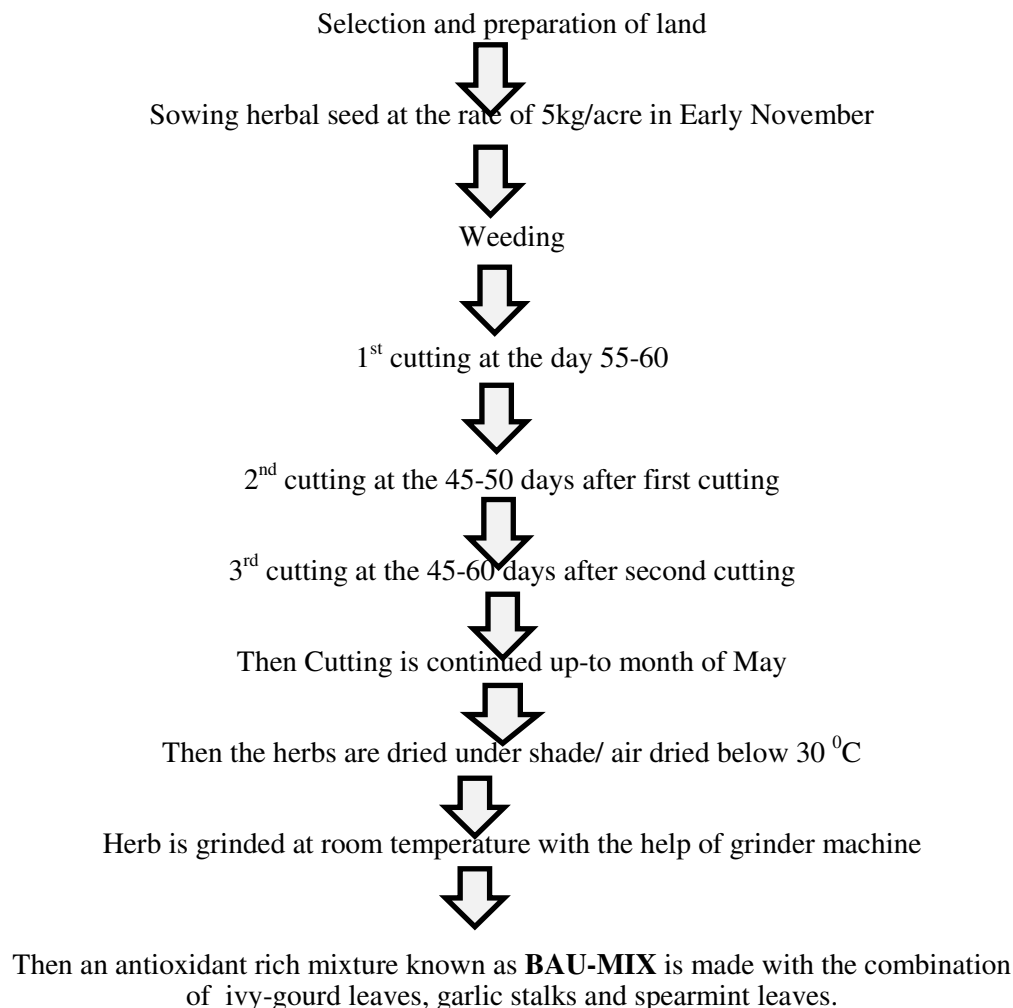
(Shuvo, 2017). It helps to improve glucose metabolism (Al-Mamun, 2008a) as well as whole body protein synthesis (Al-Mamun, 2008) by reducing heat stress due to the presence of anti-oxidative activity. Plantain accelerated the insulin sensitivity in sheep (Al-Mamun, 2017). Ivy Gourd (*Coccinia grandis*) rich in alkaloids, flavonoids, saponin, and phenol (Rodrigo et al., 2013). Leaves of the plant were reported to have anti-diabetic (Chopra et al., 1958), antibacterial activity (Girish and Satish, 2008), hepato protective activity (Rao et al., 2003). Garlic is famous for its characteristic odor, arising from allicin and other oil-soluble sulfur components (Harunobu et al., 2001). *Mentha spicata* L. commonly called spearmint used as species to enhance food flavor and consumed in small quantities. However, as they are secondary metabolism compounds that may have pharmacological activity, there is currently a growing interest in plant extracts as sources of antimicrobial and antioxidant compounds as a means of avoiding potential problems caused by excessive consumption of synthetic additives. The essential oil can contribute to the preservation of products, may develop pleasant sensory characteristics (Rodrigo et al., 2013).

Description

The total broiler chickens were divided into two groups one was control (feeding commercial feed) and another was formulated feed avoiding any sorts of antibiotic growth promoters and supplement of plantain herbs as fresh basis. Feed conversion ratio was better in plantain feeding groups. Plasma metabolites like HDL was higher in plantain feeding group compared to commercial group. Other plasma metabolites such as triglycerides, total cholesterol and LDL significantly reduced in fresh plantain group compared to commercial group. For the dose titration another feeding trial was conducted at four different groups. One group was based on formulated feed and another three groups were supplemented with 0.5%, 1%, 1.5%, fresh plantain herb on DM basis, respectively. It was concluded that 1% fresh plantain supplement was best for broiler production. Then 3rd feeding trial was conducted into two groups, one is bio-selenium supplemented and another is fresh plantain herbs supplement on DM basis. So, after completing three on station trial. Then consequently three on farm trial was conducted at gilondo village in Manikganj. The result of all trials was better in case of plantain supplement groups compared to other groups. Finally, in manikganj, plantain herbs were supplied in different form with pellet feed. The plantain formed was blenching plantain powder with pellet and shade dried plantain powder with pellet feed. The plantain supplement groups had lower mortality and better feed conversion ratio compared to commercial diet. Heme-iron, antioxidant, ω -3 fatty acid and tibia ash content were significantly higher in plantain supplementation group as plantain is rich in antioxidant, bioactive component and no antibiotics growth promoters fed to the birds. The flavor, juiciness, tenderness and taste were higher in plantain supplement group compared to commercial group and it was proved by panel test in Bangladesh Agricultural University and Manikganj. The total antioxidant capacity (TAC), and superoxide dismutases (SOD) were higher in plantain feeding group. In addition, supplementation of plantain herbs improved the quality of meat through increasing the antioxidant, ω -3 fatty acid and heme-iron level in meat. From above feeding trial it is concluded that plantain herb may be an alternative option of antibiotic growth promoters with increasing the performance index (combination of growth, feed conversion ratio and mortality). So plantain combined with other antioxidants and minerals rich and deodorized herbs like Ivy-gourd (*Coccinia grandis*), garlic (*Alium sativum*), spearmint (*Mentha spicata*) a mixture rich in antioxidant, protein synthesis enhancer, rich in minerals and capable of removing

the bad odor of the broiler meat. BAU-MIX comprises of 90% plantain, 5% ivy-gourd, 3% garlic and 2% spearmint herbs. BAU-Plaintive as an alternative to antibiotic growth promoters not only improved the quality of meat but also triggers the consumer's acceptability.

Cultivation procedure of herbs



Flow diagm. 8. Cultivation procedure of herbs.

Cultivation procedure of all herbs are same to plantain herb. In case of ivy-gourd and mint cutting is sown and in case of garlic clove is sown. No chemicals fertilizer except few amounts of urea and organic fertilizer are required.

Table 61. Standard formulae of BAU-MIX

SL.No.	Ingredients	Percentages
1.	Plantain (<i>Plantago lanceolata</i> L.) powder	90
2.	Ivy-gourd (<i>Coccinia grandis</i>) powder	5
3.	Garlic (<i>Allium sativum</i>) powder	3
4.	Spearmint (<i>Mentha spicata</i>) powder	2
Total		100

Location/ecosystem

The herbs are suitable to produce throughout the country in Bangladesh.

Benefits

- BAU-MIX improved production efficiency through reducing mortality and increasing performance index (combination of growth, feed conversion efficiency and mortality).
- BAU-MIX modulated the lipid profiles through reducing body fat, meat ether extract, total cholesterol, triglycerides, low density lipoprotein (LDL) content, and increasing the high density lipoprotein (HDL) content of broiler chicken.
- BAU-MIX accelerated the ω -3 fatty acid as well as poly unsaturated fatty acids content of broiler meat.
- BAU-MIX increased plasma total antioxidant capacity (TAC) and superoxide dismutases (SOD) are group enzymes that providing cellular defense against reactive oxygen species.
- BAU-MIX increased bone mineralization, heme-iron level of broiler meat as well as increased the meat quality in terms of better flavor, juiciness, tenderness and overall acceptability of the consumers.
- Finally, this invention would promote to use BAU-MIX, a green feed additive for broiler chicken as an alternative option to antibiotic growth promoters (AGPs) in broiler production.

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ii. Effectiveness in policy support (knowledge generation)

Component-1 (BRRI)

- Baseline survey data on daily calorie intake of street children in capital city, Dhaka reveals 27-59% deficiency of RDI (Recommended Dietary Intake) prevailed in boys population (n=243) and 28-56% gap of RDI for girls population (n=141).
- Baseline data revealed that both boys and girls for weight for age and height for age were less than -2σ score which classified as underweight and stunting population of 4-12 yrs and it is also correlated with less than 5th percentile to define shortness and low weight-for-length of our target population for both boys and girls of 4-12 yrs.
- Rice based bakery product can be prepared with nutritionally enriched rice flour. It is low cost, safe and nutritious.
- Rice-based energy dense bakery food products such as EDRB and ERDC have energy density of 5.2 and 5.0 respectively with low moisture content.
- EDRC has positive impact on street children to minimize their malnutritional status by improving their body immunity.

Component-2 (BARI)

- Baseline survey reveals that deep oil fried potato chips contained higher amount (560 – 10180 $\mu\text{g/kg}$) of toxic substance like acrylamide which is above the permissible limit as per daily intake for a person's body weight. This situation invites human health risks which can be controlled by appropriate framing of regulation strong monitoring of market and awareness of the consumers.
- SME using BARI developed small scale simplified low-cost vacuum frying machine for quality fried chips production from underutilized or low priced raw form of agricultural produces should be encouraged under present product development policy/strategy of the respective sector.
- Qualitative features of product processing (oil control, rpm of the de-oiling machine, frying temperature and time, nutritional quality affecting factors, species/ingredients combination and processing protocol information generated under the study can be designed as standard chart of chips production features.

Component-3 (BAU)

- A herbal technology using solely plantain or garlic and their mix might be used for antioxidants enriched safe mutton production;
- A herbal mixture using plantain, ivy-gourd, spearmint and garlic cloves in shade dried form at 1.0-1.5 g/kg diet might be used for the increased of egg production, and egg external and internal quality;
- A herbal mixture using plantain, ivy-gourd, spearmint and garlic cloves in shade dried form might be used for the antioxidants enriched quality meat production as an alternative to growth promoters in broiler production.
- Threshold level of plantain herb-a phyto-genic feed additive for dairy cattle was determined for the antioxidants enriched milk production in dairy cows.

- Plantain herb is safe for animal feeding and played positive role on liver health antioxidants and immune status in mouse model.
- This herbal supplementation is safe and effective for function livestock production and the products from livestock milk, meat and egg enriched with antioxidants safe for human consumption which is validated by mouse model trial.

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

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

Component-1 (BRRI)

Energy dense rice cake (EDRC) has positive impact on street children to minimize their malnutritional status by improving their body immunity.

Component-2 (BARI)

The developed vacuum frying machine and generated technology of fruits and vegetables fried chips products is a revolution for the Bangladeshi people which will ensure quality fried products.

Component-3 (BAU)

All the generated technologies mentioned in technology fact sheet are the output of lab based research. To get immediate impact the technologies must be validated, scaled-up through modification and fine tuning by piloting at farmers` level.

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

Component-1 (BRRI)

Rice based bakery product can be prepared with nutritionally enriched rice flour and sagu powder mixture. It is low cost, safe and nutritious.

Component-2 (BARI)

Different types of value-added healthy chips products from agricultural produces can be developed by further study.

Component-3 (BAU)

To the best of our knowledge this is the first time we have worked on the efficacy of medicinal plant bioactive components in diverse species (dairy cow, sheep, broiler, and layer). And finally, validated through mouse model to ensure the positive impact on human health. The novel findings would encourage the promising animal and human nutritionists to work more for the development of new technologies based on the current findings

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

Component-1 (BRRI)

Rice based bakery food products will bring a new arena in bakery industry and it will enable to set up small scale entrepreneurship in this bakery field.

Component-2 (BARI)

These technologies will help to generate new products and reduces postharvest losses of agricultural commodities, entrepreneur's development, product marketing and income generation.

Component-3 (BAU)

If the developed technologies transferred followed by piloting it would play a vital role in the production of antioxidant rich, safe and functional meat, milk and egg. Unemployed women and youth will get involved in market value chain development of herbs additives, meat and milk production, processing. Moreover, these novel technologies will create new window of entrepreneurship and improve farmers income

iv. Policy support

Component-1 (BRRI): Not applicable at this stage

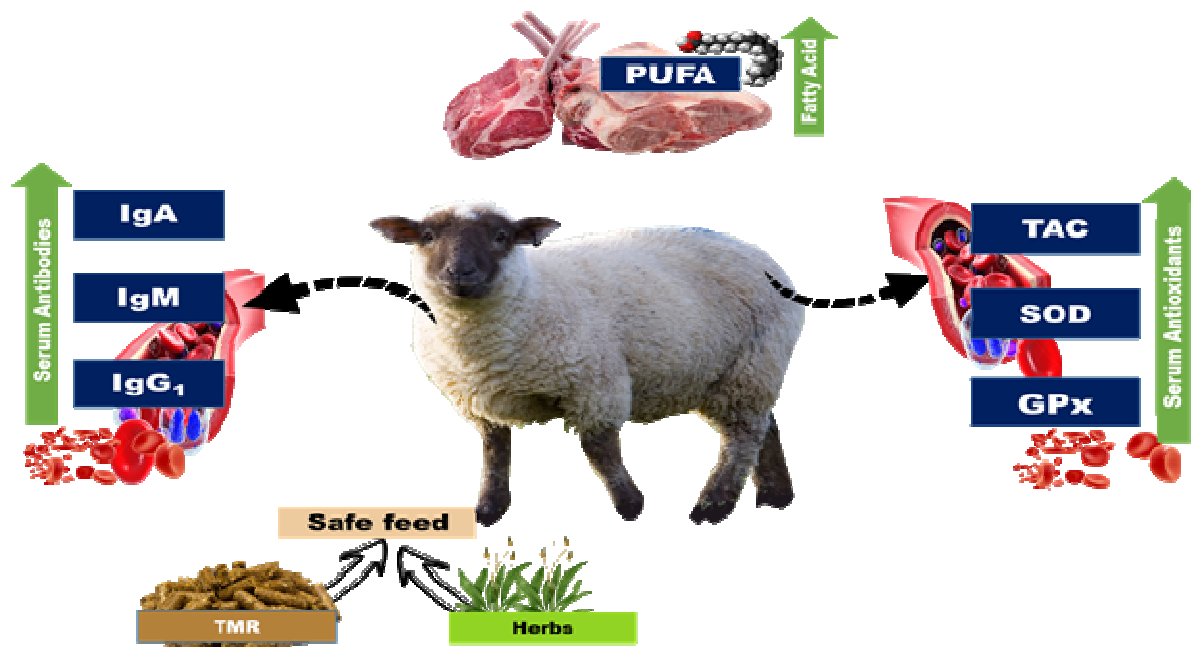
Component-2 (BARI)

BARI developed low-cost vacuum fryer will encourage the agro-processors to develop quality fried chips products which value addition in agricultural commodity. SME people are able to use vacuum frying technology for preparing different fried chips products which will increase family income and assist to alleviate poverty. Developed processing protocol of various fried chips products will motivate to the entrepreneurs using BARI developed low-cost vacuum fryer for producing nutritionally rich chips products. The developed technology will increase trust to the customers for consuming safe fried chips products. The local manufacturer can easily be fabricated in a local workshop that will add value in agro-processing industry.

Component-3 (BAU)

- *A herbal technology* using solely plantain or garlic and their mix might be used for antioxidants enriched safe mutton production.
- A herbal mixture (BAU-Plantive) using plantain, ivy-gourd, spearmint and garlic cloves in shade dried form at 1.0-1.5 g/kg layer ration might be used for the increased of egg production, egg yolk color and egg external and internal quality.

- i. A *herbal technology* using solely plantain or garlic and their mix might be used for antioxidants enriched safe mutton production.



- ii. A herbal mixture (BAU-Plantive) using plantain, ivy-gourd, spearmint and garlic cloves in shade dried form at 1.0-1.5 g/kg layer ration might be used for the increased of egg production, egg yolk color and egg external and internal quality.



- These novel herbal technologies have the capability to increase the mutton, broiler meat, egg and milk production as well as improve the level of antioxidants in that livestock products.
- Omega-3, hem iron, and low cholesterol enriched livestock products especially milk, meat and egg can be acts as antioxidants, antimicrobials and anti-aging agent that improve the immune status in human body.
- Herbal technologies may be created job opportunity for unemployed rural women which is in favour of 5th goal of sustainable development goal (SDG).
- These outstanding herbal technologies can be created job opportunity for unemployed youth thus resulting in increased the national GDP of Bangladesh (SGD's 8th goal).
- These herbal technologies can be motivated the farmers toward safe and functional livestock and food production which will ensure good health for human i.e. in favour of National Livestock Extension Policy, 2013; Animal Feed Act, 2013; The Food Safety Act, 2013 and 3rd goal of SDG.
- These super herbal technologies can be reduced the risk of antimicrobial resistance (Superbug) in Bangladesh which supports the aim of Global One Health.
- These herbal technologies might be played a vital role to reduce all sorts of degenerative disease (cancer, type-2 diabetes, alzheimer, cardiovascular diseases) by ensuring the consumption of antioxidants fortified milk, meat and egg.

I. Information regarding Desk and Field monitoring

i. Desk monitoring information of th sub project (*all cpmponents*) (description & output of consulting meeting, monitoring workshop/seminars etc.)

Desk monitoring	Date & Venue	Output
Inception Workshop	October'2018	Recommendation and comments made in the workshop for modification and improvement of the sub project document
1 st Annual Progress Review workshops	24.06.2019, BARC	Recommendation and comments on previous one year research progress
2 nd Year Half Yearly Progress Review Workshop	21.01.2020, BARC	Recommendation and comments previous one and half year research progress
2 nd Year Progress Review Workshop	27.09.2020, BARC	Recommendation and comments previous two-year research progress. Helped improvisation of research activities
Sub-project coordination meetings	24.10.19, BARC 19.02.20, BARC 18.06.20, BARC	Established effective coordination of research activities among then sub-project components
Completion Workshop	25.5.2021, BRRI	Helped evaluate the total research activity of the project

ii. Field monitoring information of th sub project (*all cpmponents*)
(date & no. of visit, name and addresses of team visit and output)

Date	No. of visit	Name	Addresses	Output
29.11.2018	01	Dr. Md. Monirul Islam Director Dr. Saleh Uddin Ahamed, Consultant	Nutrition Unit, BARC Nutrition Unit, BARC	Improvement of research activities
03.09.2019	01	Dr. Md. Monirul Islam, MD, BARC	Coordinator, PBRG sub projects, Nutrition Unit, BARC	Enhanced project activities
30.04.19	01	World Bank Mission Team	World Bank Dhaka Office	Improvement of project activities
19.12.2019	01	Mr. Mohammad Shahjahan,	Manager, PIU-BARC, NATP-2	Enhanced project activities <i>in the area of financial management</i>
31.12.2019	01	Dr. Saleh Uddin Ahmed (Consultant)	Nutrition Unit BARC, NATP-2	Improvement of research activities
02.01.2020	01	Mr. Md. Shajahan	Manager (FM) PIU, BARC, NATP-2	Improvement of financial management activities
20.09.20	01	Dr. Saleh Uddin Ahamed, Consultant, PBRG sub-projec,	Nutrition Unit, BARC	Suggested for correction and data management of research properly in record books
02.03.2021	01	Md. Matiur Rahman PD & Additional Secretary, MoA & Team Member	PMU, BARC, NATP-2	Put suggestions/comments regarding project activities
06.03.2021	01	1. Dr. Harunur Rashid,	Director PIU-BARC, NATP-2	Enhanced project activities
		2. Dr. Nowsher Ali Sarder	M&E Specialist, PIU-BARC, NATP-2	
		3. Mr. Mohammad Shahidul Islam,	Procurement Specialist, PIU-BARC, NATP-2	



Plate 25. Desk and field monitoring of sub-project activities.

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

a. Three years average information on weather for Gazipur region (2018 -2020)

Parameters	Seasons						Remarks
	Pre-Monson (Jan– April)		Monsoon (May–August)		Post Monson (Sept–December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	60	0	95.1	0	101.1	0	
Av. Temperature (^o C)	37	8.9	87.1	17.5	36.9	10.2	
Av. Humidity (%)	100	33	100	53	100	42	
Flood (year & category)			Moderate				
Av. Salinity (ppt)	0.5	0.9	0.4	0.6	0.5-	0.8	
Draught	Not reportable draught occurred during the period.						
Natural calamity	Not reportable natural calamities occurred during the period.						

Ref: Daily weather data, BRRI, Gazipur

b. Three years average information on weather for Mymensingh region (2018 -2020)

Parameters	Seasons						Remarks
	Pre-Monsoon (January – April)		Monsoon (May – August)		Post Monsoon (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	592	68	948	450	138	44	
Av. Temperature (^o C)	30	24	32	28	29	22	
Av. Humidity (%)	56	44	89	78	40	30	
Flood (year & category)	Not applicable for the area						
Av. Salinity (ppt)	0.5 – 0.8						
Draught	Not reportable draught occurred during the period.						
Natural calamity	Not reportable natural calamities occurred during the period.						

BBS report 2020 and Regional Metrologicat office, Mymensingh.

J. Sub-project Auditing (covers all types of audit performed)

1. Coordination component

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 30.10.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	52578.00	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	561029.00		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	581134.00		Financial management & project performance found satisfactory

2. Component- 1 (BRRI)

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 31.10.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	1103290.00	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	2988637.00		
Financial & Performance Audit by FAPAD on 25.10.21 for the year 2020-2021	No objection raised, found all relevant documents updated as per guideline	1639524.00		

3. Component -2 (BARI)

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub- project end	Remarks
Financial & Performance Audit by FAPAD on 18.11.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	1131370.90	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 23.12.20 for the year 2019-2020	No objection raised, found all relevant documents updated as per guideline	1763821.00		
Financial & Performance Audit by FAPAD on 25.10.21 for the year 2020-2021.	No objection raised, found all relevant documents updated as per guideline	4389841.00		

4. Component -3 (BAU)

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 25.11.219 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	2279601.00	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 17.11.2020 for the year 2019-2020.	No objection raised, found all relevant documents updated as per guideline	2394632.95		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 03.11.2021 for the year 2020-2021.	No objection raised, found all relevant documents updated as per guideline	3205518.00		Financial management & project performance found satisfactory

K. Lessons Learned

- i. Learned about the nutritional status of a segment of street children in capital city Dhaka.
- ii. Developed knowledge about formulation technique of rice-based energy dense bakery products.
- iii. EDRB and ERDC can potentially be used in minimizing malnutrition of children specially street children.
- iv. Existing status of fried chips products from processing to marketing in 08 different cities of Bangladesh.
- v. Agro-entrepreneurs and stakeholders are not aware of vacuum frying technology for processing safe fried chips products.
- vi. Most of the people are unknown about hazards, hygienic practices and healthiness.
- vii. Slices of fruits and vegetables having high sugar content require optimum frying temperature with time to reduce browning and toxic substances.
- viii. De-oiling plays an important role to reduce free fatty acid and rancidity of the fried chips product.
- ix. As superbug or antimicrobial resistances has become persistent in human body due to injudicious use of antibiotics in livestock industry in the past decades. Farmers are searching alternative technology for better, and antioxidants fortified livestock production with health benefits.
- x. Application of herbal technology in livestock sector of Bangladesh is increasing tremendously due to its safe, economic and antioxidants fortified milk, meat, and egg production.
- xi. Optimum level of herbal supplementation improved the performance of livestock as well as level of antioxidants in livestock product like milk, meat, and eggs.
- xii. Convincing power and adaption capacity is the most crucial point for the success of on-farm feeding trail.

L. Challenges (if any)

- i. Managing the physical challenges like dealing with street children in hostile situation in different places of Dhaka city while conducting baseline survey and during impact study of EDRC for keeping street children in one designated place for certain 4 months' time for one hour free schooling by incorporating experienced researchers, academicians and data enumerator and social workers.
- ii. Most of the traders and stakeholders are not known regarding vacuum frying technology so it was too challenge for us to fabricate a low-cost vacuum fryer in a local workshop and introduce to the agro-processors at all levels.
- iii. Information collection from baseline in different selected locations about chips processing and marketing most of the respondents are not willing to talk because of hygienic condition and frying practice are not maintained properly by the processors.
- iv. It was difficult to optimize the processing protocol using locally fabricated BARI Vacuum Fryer within short time to prepare fried chips and evaluating the shelf life of the products due to covid-19 pandemic situation happening in the country.
- v. It was really challenging for us to adopt the herbal technology at field level due to following reasons:

- Few farmers are less interested to take new or herbal technology, as there not concern about benefit of herbal technology or either they are less educated or uneducated.
- Proper feed consumption recording for dairy, broiler and layer trial were slightly laborious as stayed with farm during the trial period is difficult.
- Data collection and management are very complicated though in our research it was strictly maintained.
- Supplementation of herbs powder with basal ration is required extra labor but addition of herbal powder with pellet diet during feed formulation might be reduced the cost of labor.
- As the sub-project, as per agreed plan, was running during the pandemic COVID-19, thus we faced lot of problems during feed, vaccine and other supporting materials procurement and even in sample analytical activities.

M. Suggestions for Future Planning (if any)

- i. EDRB and EDRC can potentially be used in school feeding nutrition program and disaster mangement including refugee crisis, flood or cyclone related emergency relief operation program to mitigate malnutrition and emergency response of energy dense dry food as suppliments.
- ii. For food and nutritional security, vacuum frying technology has potential role for preparing quality fried chips products from different agricultural produces. BARI developed low-cost vacuum fryer can be used conveniently in SME level. This novel technology is used commercially in many countries to prepare vacuum fried chips which provides unique appearace with texture. The technology is new in Bangladesh therefore, it is required to introduce around the country and validate at stakeholders' level as well as agro-processing sector for disseminating the information through appropriately piloting or up-scaling activities. BARI component urge to PIU, BARC, NATP 2 Phase for incorporating this technology as field validation so that agro-processors will be interested and benefitee to use the technology for preparing toxic free safe and quality fried chips products for domestic consumption as well as export purpose.
- iii. Through the present research activities under the sub-project, the BAU component developed herbal technologies for safe and antioxidants fortified livestock production. In the field of animal nutrition, these novel herbal technologies offer promising opportunities for the animal scientists for future research, government policy makers for the safe and functional animal production guidelines, feed industries to formulate value added ration for the animal. It is highly recommended that the safe and verified plantain herb technology for antioxidant-enriched mutton production in sheep might be disseminated among farmers/animal producers through validation, medication, fine tuning, and up-scaling at a greater scale in field level.
- iv. Remaining research under the hypothesis on the antioxidant properties of plantain may influence the hepatic macrophages or other immune cell population in the liver may be included in future research plan.

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Signature of the Coordinator  (Dr. Md. Monirul Islam) Date: 15.10.2021 <i>Member Director (Fisheries)</i> <i>Bangladesh Agricultural Research Council</i>	Counter signature of the Head of the organization/authorized representative  (Dr. Shaikh Mohammad Bokhtiar) Date: 15.10.2021 <i>Executive Chairman</i> <i>Bangladesh Agricultural Research Council</i>
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Appendices

Coordination component (BARC)

Appendix – BARC: A

Recommendation of the inception workshop and status of action taken

Recommendations	Action Taken
General comment Reframe the title of the program that reflects the total research works on standardization of human nutrition level standards and quality livestock feed production activities of the component projects as whole	Titled renamed as “Fortification and standardization of nutritional level in selected human food and efficacy test of polyphenolic compounds in livestock products”.
Component 1 (BRII)	
Title and goal of the program are not presented properly. Necessary corrections suggested;	Necessary corrections done
Re-write objective “1” of the component as “To know the dietary pattern of street children in the capital city of Dhaka” instead of “Survey on dietary City, Dhaka”;	Changed
Re-write objective “3” of the component as “ Impact of formulated rice based food products on street children” instead of “Impact evaluation studies onstreet children”;	Changed
Specify the meal source in the questionnaire (e.g. Own prepared, purchased, others etc);	Source of meal specified
Sources of ingredients like protein, carbohydrates and fat to be specify for developing energy dense food;	Shown in the text
Outputs and outcomes of the project are not stated properly. Outputs will present the result/production of the work while the outcome will be the ultimate broader aspect of impact of the result/production;	Stated as per suggestion
Component 2 (BARI)	
Re-write the program title;	Done
Specify the name of value added products to be produced;	Mentioned as per suggestion
Objective no. “3” of the project (enhancement/fortification) can be omitted ;OR partial work of same nature can be carried out under activities of objective “2”; .	Change made
Inclusion of work on identification of standard packaging materials and methodology for the preservation of products suggested;	Included as per suggestion
Component 3 (BAU)	

Outputs and outcomes of the project are not stated properly. Outputs will present the result/production of the work while the outcome will be the ultimate broader aspect of impact of the result/production;	Stated as per suggestion
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Annexure – BARC: B

Recommendation of the half yearly workshops

Recommendations of the First Half Yearly Workshop	Action taken
Component 1 (BRRI)	
Standard deviations of various parameters data need to be expressed to focus the range and extend of magnitude;	Followed accordingly
Clear indication of relationship of dietary intake/nutrition level among the factors like age group, sex, income in the baseline information to be focused;	Shown in respective parts of the reports
Name of the varieties of rice use for energy dense food preparation should be specified;	Specify in the report
Component 2 (BARI)	
For project interest, immediate completion of procurement of goods urged;	In-time procurement of goods done
Organizing of more training in future under the project for popularization of fabricated machine and its operation procedure suggested	Action taken
Benefit-cost analysis of production process should be taken into account;	Applied where necessary
Attempt should be made to retain the original flavour of the fruit sources in the products;	Measures taken
Component 3 (BAU)	
Considering the nature of the research, though a baseline not required, but to justify the study findings. Suggested to review of sufficient related previous studies/literature/information and to incorporate in the reports to be presented	Literature reviewed adequately

Appendix – BARC: C**Recommendation of the Annual workshop**

Recommendations of the First Annual Workshop	Action taken
Component 1 (BRRI)	
• Urges to complete baseline study within next one month;	Completed as per work plan
• Name of anti-oxidants are to be shown in the report;	Shown in report
• Causes of calorie gap between observed and reference values in case of male and female members should be explained in the report;	Causes mentioned in the report
• Acceptable cost benefit result should be include in the final report;	Not applicable
• Valid test for biological value of food (nutrition) and enriched food should be done;	Covered under the research
Component 2 (BARI)	
• Shelf life of products, safety aspects and maintaining of quality should be ensured under the same research coverage;	Research addressed all the areas
• Category (variety) of local jack fruits, various development stages of fruits and their nutrients level etc should be considered for study their suitability for chips production;	It was not possible to comply the recommendation because of shortage of fund and time
Component 3 (BAU)	
• Threshold level of anti-oxidants in herbal additives should be determined;	Covered under the present research
Recommendations of the Second Annual Workshop	Action taken
Component 1 (BRRI)	
• Detail result of the project activities was not presented that hampered true review exercises;	Followed properly in the next review workshops
• State of present activity for impact study of rice based food on street children's were not clearly understandable which is one of the vital part of the research;	This was properly discussed in the project document
Component 2 (BARI)	
• Storage study of the of vacuum fried sweet potato and jackfruit chips are to be completed immediately	Necessary step taken in-time
• Benefit and cost analysis of the production process should be taken into account. Suggested to enclose a separate analysis sheet at the end of the respective chapter of the final report;	It is not included in the original proposal, but analysis done where necessary
Component 3 (BAU)	
• No specific comment	

Appendix – BARC:D**Recommendation of the coordination meetings**

Central Coordination meeting at BARC	
Recommendations	Action taken
To ascertain better product quality, it is suggested to increase the processing temperature of vacuum frying machine within the range 120 – 140⁰ C by the BARI component;	Experiment done at various possible temperature
Clarification and justification of the findings/results of analysis of efficacy of polyphenolic compounds should be discussed in the next reports of BAU component;	Properly discussed in the next reports
Sincere attention for avoiding duplication of similar research implementation by the same component with financial support from different sources is completely unethical. To facilitate ideal individual research, it is better to select and develop new areas within the available capacities;	Paid proper attention to avoid duplication of works
Progress is poor as per work plan need to speed up;	All efforts given, but disease epidemic delayed fund release and causes slower progress in several stages of implementation
Two other virtual Coordination meetings	
Component 1 (BRRI)	
The present impact study on street children on rice based nutraceutical food items should be presented clearly and specifically focusing the variation of each study parameters with that of the respective base line information at the end;	Done accordingly
Immediate effort should be taken with the cooperation of local Civil Surgeon/ health departmental officials for selection of areas and clients for impact study of selected rice based product to evaluate the dietary intake level, health and growth;	Cooperation ensured by the other agencies
Necessary safety measure should be followed strictly by the field staffs while collecting sample and data from the selected areas of street children on nutrition intake and impact study;	Followed properly
Delay in fund release hampering completion of procurement process that hindering research progress, attention of PIU solicited;	Necessary steps taken by the coordination unit
Component 2 (BARI)	
Progress of research are in right track;	

• Stage of development of fruit while using and its impact on product quality, packaging and shelf life should have clear indications;	Taken care of the matter
• Special emphasize to temperature, time length of treatment, humidity, oil quality, vacuuming time and pressure should be taken into consideration during product development processing and packaging;	All species taken into consideration
• Prompt decision on revision of project and reshuffle of funding allocation as per prevailing field and laboratory requirement from the competent authority;	Coordination unit took necessary action in consultation with the PIU authority
• Product development protocols are to be specific and self-explanatory that should present through flow charts;	Designed accordingly
• Repetition of few works on packaging materials and shelf life where necessary;	Done
At least two more training suggested on product development and preservation in the remaining project period;	Ensured
Component 3 (BAU)	
• Remaining analysis of milk and eggs under objective no. 2 of the project are to be completed immediately;	Completed as per plan
• Necessary funding support from PIU (as per project provision) for procurement of chemicals and birds should be given immediately (where the RFQ process is almost completed);	Necessary steps taken from the coordination unit
• Causes of experimental sheep death with supporting postmortem information are to be provided to the Coordinator stating research difficulties (if arises any);	Instruction followed
• Full pledged base line summary to be added while submitting next report;	Added in the next reports.



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