Project ID: 719

Competitive Research Grant (CRG)

Sub-Project Completion Report

On
Production of low cholesterol healthy mutton by using natural herbs

Project Duration
July 2017 to September 2018

Department of Animal Nutrition, Bangladesh Agricultural University
Mymensingh

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Project Implementation Unit
National agricultural Technological Program-Phase II (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka-1215
Bangladesh

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Published in: September 2018

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Acronyms

<	= Less than
>	= Greater than
^{0}C	= Degree Celsius
ADP	= Adenosine di-phosphate
ALT	= Alanine transaminase
AST	= Aspartate transaminase
ATP	= Adenosine tri- phosphate
B.W	= Body weight
BARC	= Bangladesh Agricultural Research Council
BUN	= Blood urea nitrogen
CF	= Crude Fiber
CK	= Creatine Kinase
CL-diet	=Control diet
Co-PI	= Co-Principal Investigator
CP	= Crude protein
CRG	= Competitive Research Grant
DCF	= Digestible crude fiber
DCP	= Digestible Crude protein
DEE	= Digestible ether extracts
DM	= Dry matter
EE	= Ether extract
FCR	= Feed conversion ratio
gm	= gram
GL-diet	= Garlic leaf diet
GPx	= Glutathione Peroxidase
Hb	= Hemoglobin
HDL	= High density lipoprotein
IgA	= Immunoglobulin A
IgG	= Immunoglobulin G
IgM	= Immunoglobulin M
LDL	= Low density lipoprotein
ml	= milliliter
NATP	= National Agricultural Technology program
PCr	= Phosphocreatine
PG-diet	= Plantain + garlic leaf diet
PI	= Principal Investigator
PL-diet	= Plantain diet
RBC	= Red Blood Cell
SEM	= Standard Error Mean
SOD	= Superoxide dismutase
TAC	= Total antioxidant capacity
TDN	= Total digestible nutrients
TG	= Triglycerides
Tk.	= Taka
TMR	= Total mixed ration
TSH	= Thyroid stimulant hormone
USAID	= United States Agency for International Development
VLDL	= Very low density lipoprotein
WBC	= White Blood Cell

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Executive Summary

The present experiment was conducted to investigate the effect of supplementation of plantain (*Plantago lanceolata* L.) and garlic leaf (*Allium sativum*) with Total missed ration (TMR) pellet on growth performance, plasma metabolites including the level of antioxidant and cholesterol and carcass trait in sheep. Thirty two local sheep (*Ovis aries L*), aged around one year with the body weight of 9.1±0.2 kg were used to conduct the experiment. The animals were grouped into four with each group having eight animals. For all the dietary treatment groups, TMR pellet were offered to keep around 1.5 times maintenance level (NRC, 1985). For the plantain supplemented group (PL-diet) 10 gm of the dry mater (DM) of plantain and for garlic supplemented group (GL-diet) 10 gm DM of garlic leaves supplementation was supplied daily to the sheep. In case of mixed herbs supplemented group (PG-diet) 5 gm DM plantain and 5 gm DM garlic leaves were supplemented daily to the experimental sheep. Supplementation of different herbs with TMR pellet had no significant effect ($P < 0.05$) on digestible crude protein, digestible crude fiber and digestible ether extract on sheep. But digestible nitrogen free extract and total digestible nutrient were significantly higher ($P < 0.05$) in plantain supplemented group (PL-diet). Treatment groups however exhibited higher daily weight gain compared to control group (CL-diet). Daily DM and crude protein (CP) intake were highest for PL-diet group which was similar to PG-diet group compared to other treatment groups. Lowest feed conversion ratio (FCR) was found in PL-diet group compared to other treatment groups. Higher plasma glucose and triglycerides were found in control group (CL-diet) and garlic leaf supplemented group (GL-diet) compared to other groups. Blood urea nitrogen (BUN) was higher both in plantain supplemented group (PL-diet) and mixed herbs supplemented group (PG-diet) compared to control and garlic supplemented groups. Lowest plasma total cholesterol and LDL-cholesterol were found in plantain and garlic supplemented group compared to control and mixed herbs supplemented group. Herbal supplementation also exhibited positive impact to raise serum albumin and globulin concentration. Higher concentration of immunoglobulin G1, immunoglobulin G2, immunoglobulin M, immunoglobulin A were found in PL-diet group followed by mixed herbs supplemented group (PG-diet), garlic leaf supplemented group (GL-diet) with the lowest value for control group (CL-diet). On the other hand, herbal supplementation with TMR pellet had no significant effect ($P < 0.05$) on growth hormone (GH), insulin, glucagon and thyroid stimulating hormone level (TSH) in sheep. But, a positive tendency was found between herbal supplemented groups. Lowest alanine transaminase (ALT) and creatine kinase activities were found in plantain supplemented group (PL-diet) compared to other treatment groups. Highest serum free radicle scavenging capacity was found in plantain supplemented group (PL-diet) and then mixed herbs supplemented group (PG-diet) and garlic leaf (GL-diet) compared to control group. There was no significant difference ($P < 0.05$) in carcass characteristics among the treatment groups rather than caul fat, pelvic fat and dressing percentage. Caul fat and pelvic fat were lowest in plantain supplemented group (PL-diet) followed by mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet). Highest dressing percentage was found in PL-diet group. Mutton ether extract content was significantly ($P<0.05$) lower in all herbal supplementation group compared to control group. Dripped loss and cooking loss had no significant difference but higher rib eye area was exhibited in plantain supplemented group (PL-diet) followed by mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet). There was a significant difference in saturated fatty acid (SFA) and unsaturated fatty acid (USFA) among the dietary treatment groups. SFA content was significantly ($P<0.05$) lower in all herbal supplementation group compared to control group and vice versa for USFA. Monounsaturated fatty acid (MUFA) was lower ($P<0.05$) in all herbal supplemented group compared to control. Linoleic acid, major interest for producing functional meat was highest in plantain supplemented group (PL-diet) followed by mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet). Finally it could be concluded that plantain-diet and mixture of plantain-garlic diet might be used for the production of healthy, lean and functional mutton in Bangladesh.

CRG Sub-Project Completion Report (PCR)

A. Sub-Project Description

1. **Title of CRG sub-project :**

   Production of low cholesterol healthy mutton by using natural herbs

2. **Implementing organization**:
   Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh.

3. **Name and Full address with phone and E-mail of Principal Investigator (PI)**

   Principal Investigator:
   Dr. Mohammad Al-Mamun
   Professor, Department of Animal Nutrition
   Bangladesh Agricultural University, Mymensingh-2202
   Cell: 01715-051093; E-mail: mamunshimu@yahoo.com

4. **Sub-project budget (Tk) :**
   4.1 Total: 4508972.00
   4.2 Revised (if any):

5. **Duration of the sub-project:**

   Start date (based on LOA signed) : 19 July 2017
   End date : 30 September 2018

6. **Justification of undertaking the sub-project:**

Feed additives are the products used in animal nutrition for the purposes of improving the quality of feeds, animal production performance and health, quality of animal products, consumer health and decreasing environmental impact of animal production (Nuntavan, 2012). During the last five decades, chemicals were widely used as feed additives especially antibiotics as growth promoter (Al-Mamun *et al*., 2008). The residues from antibiotics in animal products and resistance to antimicrobial agents in human have been reported which lead to antibiotic banning as growth promoter in the animal feeds since January 1st, 2006 (Al-Mamun *et al*., 2008). The EU banning policy accelerated the search for the alternative feed additives. Natural herbs are the leading alternatives that exert several biological activities beneficial to animal production. Natural herbs have been evaluated for their ability to alter ruminal fermentation and improve nutrient utilization in ruminants (Wang *et al*., 2000; Greathead, 2003). In consequence, some well-known herbs; Plantain (*Plantago lanceolata*), and Garlic (*Allium sativum*) have been used in ruminant feeding and found positive impact on plasma nutrients metabolism and improving propionate production (Al-Mamun *et al.*, 2007; 2008b; Kamruzzaman *et al*., 2011). Moreover, a growing awareness among consumers to consume antioxidant-rich foods has provided opportunities for the livestock industry to focus on production of antioxidant-rich milk and meat or functional foods (Chauhan *et al*., 2014). Therefore, the present research was designed to determine the effect of natural herbs, plantain and garlic on mutton antioxidant function and cholesterol content in sheep.

7. Sub-project goal:

Safe and quality sheep production using natural herbs

8. Sub-project objectives:

- To test the efficacy of medicinal herbs on the growth performances of sheep
- To identify the level of antioxidant and cholesterol in the meat from experimental sheep

9. Implementing location:

Shahjalal Animal Nutrition Field Laboratory, Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh.

10. Methodology in brief:

Approach:

The experiment was conducted for 90 days with 32 local sheep to study the effect of plantain (*Plantago lanceolata* L.), garlic leaf (*Allium sativum*) and mixed herbs (combination of plantain and garlic leaf) on growth performance, plasma metabolites, carcass and mutton quality. The total experimental procedures, animal handling, blood and meat sample collection procedure were reviewed and approved by the animal care committee of Bangladesh Agricultural University Research System (approval no. BAURES/ESRC/2019/AH/03).

Experimental sheep and place

Thirty two indigenous sheep (*Ovis aries L*) almost similar in age (around one year) and average weight (9.1±0.22 kg) were purchased from Muktagacha, Mymensingh and Modhupur, Tangail. Feeding trial of sheep was conducted at Shahjalal Animal Nutrition Field Laboratory under the Department of Animal Nutrition, Bangladesh Agricultural University (BAU), Mymensingh.

10. 1. Testing the efficacy of medicinal herbs on the growth performances of sheep

Experimental design

The animals were divided into four groups having eight animals in each group using completely randomized block design (Table 1.). Different dietary treatments were categorized as follow:

CL-diet: Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm)

PL-diet: CL-diet + 10 gram DM Plantain supplementation

GL-diet: CL-diet + 10 gram DM garlic leaf supplementation

PG-diet: CL-diet + 5 gram DM plantain and 5 gram DM garlic leaf supplementation.

Table 1: Layout of the experiment design showing distribution of sheep

Replication	Treatment groups				Total
	CL-diet	PL-diet	GL-diet	PG-diet	
R1	1	1	1	1	4
R2	1	1	1	1	4
R3	1	1	1	1	4
R4	1	1	1	1	4
R5	1	1	1	1	4
R6	1	1	1	1	4
R7	1	1	1	1	4
R8	1	1	1	1	4
Total	8	8	8	8	32

*(**CL-diet:** Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm), **PL-diet:** CL-diet + 10g DM Plantain supplementation; **GL-diet:** CL-diet + 10g DM garlic leaf supplementation; **PG-diet:** CL-diet + 5g DM plantain and 5g DM garlic leaf supplementation.)*

Plantain (*Plantago lanceolata L.*) and Garlic (*Allium sativum*) cultivation

Two rectangular 36m^2 area land with sufficient sunlight exposure was selected for cultivating the plantain and garlic in the Sahajalal Animal Nutrition Field Laboratory, BAU, Mymensingh (Plate-1). Firstly the land was prepared by ploughing and harrowing. The land was divided into 6 plots for both plantain and garlic. The area of each plot was about 6m^2 (3m × 2m). No chemical fertilizer was used with a purpose to produce safe and functional food. Plantain seeds were bought from New Zealand and garlic bulb was brought from local market.

Plate 1: Cultivated land of plantain (*Plantago lanceolata L.*) and garlic (*Allium sativum*) at Shahjalal Animal Nutrition Field Laboratory, BAU.

Experimental diet

Total mixed ration (TMR) pellet diet was supplied to all groups of animals. The TMR pellet was prepared using straw, wheat, road side grass, molasses and salt (ME 2390 kcal/kg dry matter, 15.07% crude protein). In all dietary treatment group TMR pellet was offered to keep around 1.5 times maintenance level (NRC, 1985). In plantain supplemented group (PL-diet group) 10g DM of Plantain was supplied to the sheep daily, similar strategy was followed for the garlic supplemented group (GL-diet**)** that was 10g DM garlic leaves supplementation; in case of mixed group 5g DM Plantain and 5g DM garlic leaves were supplemented to the experimental sheep daily.

TMR pellet preparation

All the ingredients required for pellet preparation were collected from local market and before grinding the grass and straw were dried sufficiently. After grinding the grass, straw and mustard oil

cake all the ingredients were mixed well using mixer machine (Plate 2), during the time of mixing 10% water was added with the ingredients and then pellet was prepared in pelleting machine (Plate 2) using 6 mm pore size dice.

Plate 2: Mixture machine (left); Pelleting machine (right)

Table 2: Ration formulation and TMR chemical composition

Ingredients	Amounts (%)
Dry grass	27
Rice straw	16
Wheat bran	30
Mustard oil cake	15
Molasses	11.5
Common salt	0.5
Calculated chemical composition of pellet (%)	
Dry matter	90.38±0.5
Crude protein	15.07±0.07
Crude fiber	18.82±0.1
Ether extract	3.03±0.12
Ash	10.48±0.34
Nitrogen free extract	52.6±0.26
ME[1], MJ/kg DM	2390

[1]*ME was determined by using mathematical model adopted from NRC (2006).*

Maintenance and managerial operation

Animal cage

The sheep shed was to the west of the Shahjalal Animal Nutrition Field Laboratory. Sheep were reared separately in cages with an area of about 0.91 m^2 (120cm × 76cm) for each cages. Before keeping the lambs in the cage the cages were cleaned with disinfectant and removed all type of rubbish and filth. Plastic bucket was used for supplying water for the animals and feed was supplied in the feeder.

Temperature and ventilation

The side wall of the cages was exposed to grill. So that air and light could pass uninterruptedly to maintain the normal atmospheric temperature to about 25±4^{0}C in the house. The temperature of the lambs was measured recurrently with the clinical thermometer. The range of rectal temperature was recorded at regular interval (about 101 – 102^{0}F). Facility was developed to protect the animals during heavy rain or excessive heat.

Feed and water supply

The TMR pellet was supplied to the animals twice in a day. Daily required amount of total TMR pellet was divided in two parts, one part was supplied in the morning (8.00 am) and another part was supplied in the afternoon (4.00 pm). Clean and fresh water was provided *ad libitum* basis per day. No growth promoter, antibiotics or feed additives was provided to the lambs.

Bio-security

Strict bio-security was maintained during the whole feeding trial. Water mixing with potassium permanganate was kept in a tray at the entrance of the stall. Proper mask, apron, hand gloves were used during handling the lambs, feeding and watering to the lambs, collecting sample from the animal and cleaning the stall. Each time the feeder and drinker was cleaned and washed with aseptol detergent before giving feed and water to the animal.

Deworming

Lambs were dewormed with moxidectin (Cydectin, Fort Dodge Animal Health) at a dosage of 0.2 mg/kg of body weight.

Health check and Clinical observation

The lambs were observed twice a day for clinical sign such as any type of lesion, mouth discharge, slow movement, infrequent sitting, lack of appetite and any other abnormalities from normal behavior.

Growth performance record

Lambs were weighed at the onset of trial and then at the interval of one week throughout the experimental period. The average body weight gain of lamb was calculated by deducting initial body weight from the final body weight. The final body weight gain was measured just prior to slaughtering.

10.2. Determination of the level of antioxidant and cholesterol in the meat from experimental sheep

Determination of plasma metabolites

Collection of blood samples

Blood samples (5 ml each) were collected on day 89 of the feeding trial in a heparinized tube and kept in an ice box until centrifugation. Samples were centrifuged at 10000 × g at 4^{o}C for 10 minutes for plasma separation and plasma was stored at -20^{o}C pending analysis.

Analysis of blood samples

Analysis of blood plasma for measuring the concentration of plasma glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL- cholesterol, VLDL- cholesterol in blood were performed using different enzymatic kits according to Toda *et al*. (1985) at Animal Nutrition Laboratory.

Collection and analysis of urine and fecal samples

Urine and feces were collected every day until last 7 days of experimental period. Urine was separately collected by using 1 mm perforated net in a bucket having 50 ml 6 N H_2SO_4 to prevent unwanted vaporization of ammonia from urine. Fecal samples were collected on the wired net try. After collection, urine samples were stored at -20^{0}C for analysis and the fecal samples were dried at 105^{o}C in hot air oven for 24 hours. Then the fecal samples were ground to 1 mm mesh and stored at -20^o C for further analysis. Proximate components of feces and urine samples were analyzed according to AOAC (1995).

Analysis for meat quality

Following activities and measurements were performed to evaluate the meat quality:

Slaughtering, processing and measurements

To measure the meat quality and carcass characteristics lambs were slaughtered. The lambs were fasted for whole night. Live weights of each lamb were recorded prior to slaughter. The animals were slaughtered according to standard method. By this method lambs were bled by cutting throat and then slaughtered by severing the head at its articulation on the occipito-atlantal space. The conventional flaying method was followed (Plate-3). At the time of bleeding the blood was gathered in a pail. The thorax of the sheep was pressed sufficiently. The amount of blood was recorded. Slaughtered lamb was also weighed after complete bleeding. Forelimbs and hind limbs were separated and weighed. The head was removed and weighted. Weight of skin was recorded. After separating the stomach as well as the gut, they were weighed. Then after removing the fill gut and stomach, the empty stomach and gut were weighed. The weight of caul fat was recorded. Heart, spleen, liver, kidney, lungs + trachea were removed and weighed separately. Heart fat, kidney fat and pelvic fat were also measured to determine the fat percentage of the animal. The hot carcass weight was measured after complete dressing and evisceration (Plate-4). The weight of neck, leg was also recorded.

Plate 3: Flaying of sheep

Plate 4: Carcass of Sheep

Determination of meat quality and carcass parameters

- ***Dressing percentage***

Dressing percentage of the slaughtered animal was estimated by using the following formula:

$$\text{Dressing percentage (\%)} = \frac{\text{Weight of the hot carcass weight}}{\text{Live weight during slaughtering}} \times 100$$

- ***Eye muscle area (EMA)***

To measure Eye muscle area the hot carcass was split between 13^{th} and 14^{th} ribs. From the cross section the area was traced three times onto an acetate paper. Then from the weight-area relationship of the acetate paper the average area of each single 'eye muscle' was estimated.

$$\text{Eye muscle area (\%)} = \frac{\text{Weight of acetate paper for total eye muscle area}}{\text{Weight of acetate paper for one cm square}}$$

Collection of meat samples

Meat samples for chemical analyses i.e. proximate composition and for determination of p^H, drip loss and cooking loss were taken from thigh region of each slaughtered sheep. Meat sample approximately 10g for proximate composition, 50g for drip loss and 10g of meat sample for cooking loss determination were taken from thigh region of each animal. The meat samples were weighed carefully and packed with marked separately.

Drip loss measurements

Each sample (weighing about 50g) was left hanging within an inflated plastic box (4^0C) for 24 hours. The percentage of drip loss was measured as described by Joo*et al*. (1995).

The drip loss was estimated by using the following formula:

$$\text{Drip loss (\%)} = \frac{\text{Weightof meatsample} - \text{Weightof dry meatsample after 24 hrs}}{\text{Weightof meatsample}} \times 100$$

Cooking loss measurements

Meat samples weighing about 10g were boiled to an internal temperature of hot water bath at 90^0C for 30 minutes followed by surface drying and weighed. Cooking loss was determined by expressing cooked sample weight as a percentage of precooked sample weight following the procedure of Aaslyng *et al*. (2001).

$$\text{Cooking loss (\%)} = \frac{\text{Weight of precookedsample} - \text{Weight of cookedsample}}{\text{Weight of precookedsample}} \times 100$$

Proximate components of meat

Dry matter

Meat sample about 5g was taken into the crucible. Then it was heated up to 105^0C in an electric oven for 24 hours. It was done in accordance with the (AOAC, 2005). All determination was done in double and the mean value was recorded.

Crude protein

Crude protein was determined by Kjeldahl method (Salo-vaananen *et al*., 1996) as for procedure shown in the following diagram:

5 gram sample +1.5 gm catalizer mixer (50gm K_2SO_4 + 5 gm $CuSO_4$ + 0.5 gm selenium powder) in a kjeldahl flask

↓

20 ml concentrated H_2SO_4 taken in the flask. Set the flask in digestion unit and heated 420^0C for 45 minutes. If the sample is not green color, heat additional 15 minutes.

↓

Cooling for half an hour and add 80 ml distilled water and 32 ml NaOH Solution

↓

Five minutes collection with 20 ml boric acid /0.4 gram

↓

Then titration with 0.1N HCl (up to pink color)

The correspondence formula to determine % nitrogen requirement is:

$$\frac{\text{Titrate required (ml)} \times 0.014 \text{ (milli-equivalent of } N_2) \times \text{Strength of HCl} \times 100}{\text{Weight of sample}}$$

Again, % of CP = % of nitrogen × conversion factor (6.25)

Ether Extract

Ether extract content was determined by Soxhlet apparatus using diethyl ether. At first flask weight was taken. Then about 2 gm sample was taken in a thimble and added 200 ml acetone in a Soxhlet. Extraction was done at 40-45°C which took about 7-8 hours. After extraction the flask were taken out and dried in oven for 30 minutes at 100°C. The flask containing ether extract was cooled in a desiccator and weighed. The calculated value for ether extract content was obtained as percent of the sample.

The formula is mentioned below:

$$\% \text{ of ether extract} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

Moisture

The moisture which would be lost can be estimated from the pre-weighed porcelain crucible in an electric oven at 105°C for about 24 hours until constant weight was obtained and the moisture content of the sample was determined according to the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight of wet meat} - \text{Weight of dry meat}}{\text{Initial weight of wet meat}} \times 100$$

Crude Fiber

For crude fiber determination about 2 gm sample was boiled with 125 ml H_2SO_4 (1.25%) for 30 minutes. Filtering was done to free the acid and the residue was boiled with 1.25% NaOH for 30 minutes. Then this residue was dried in oven drier at 105^{o}C for 24 hours and after that for ignition this was placed in a muffle furnace within a crucible at 600^{o}C for 4-5 hours. Then the weight was taken as ash regarded.

$$\text{Crude Fiber (\%)} = \frac{\text{Waste (residue) dried weight} - (\text{cruicible} + \text{ash}) \text{ weight}}{\text{weight of the sample}} \times 100$$

Mutton cholesterol determination

About 2 gm of each sample were saponified according to a modified version of the method described by Stewart *et al.* (1992), with 4 ml of 50% potassium hydroxide and 6 ml of 95% absolute ethanol heated for complete solubilization at 40ºC, and then heated for 10 minutes at 60ºC. After this, 5 ml of water were added and the samples were cooled. The non- saponifiable fraction was extracted three times using 10 ml of hexane. Aliquots of hexane extracts (3 ml) were dried under a nitrogen flow. After saponification, samples were analyzed by high-performance liquid chromatography methods followed by Jussara *el al*. (2006).

Mutton 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 2.25mm ID. The operating condition was programmed at oven temperature 150^{0}C (holding time 5 min), 8^{0}C/min –190^{0}C (holding time 0 min), 2^{0}C/min -200 ^{0}C (holding time 10 min), injection port temperature and detector temperature were 250^{0}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 ^{0}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 C_4-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^{0}C) in a series of screw capped test tubes. Aliquots of 1µL 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.

Statistical analysis

Data were represented as the mean ± SD (standard deviation). All data were subjected to one-way ANOVA, and the significance of difference among means was determined using Tukey's HSD test (1953) and differences at $P<0.05$ were considered statistically significant.

All analyses were conducted in SPSS (2002) by using following statistical model.

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

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

11. Results & discussion

11.1. Testing the efficacy of medicinal herbs on the growth performances of sheep:

Nutrients digestibility

Supplementation of different herbs with TMR pellet found to have no significant effect (P <0.05) on digestible crude protein, digestible crude fiber and digestible ether extract on sheep (Figure 1). But digestible nitrogen free extract was significantly higher (P < 0.05) in plantain supplementing group (PL-diet) and then chronologically showed the following consequence plantain and garlic leaf mixed herbs supplemented group (PG-diet group), garlic leaf supplemented group (GL-diet group) compared to control group (CL-diet group).

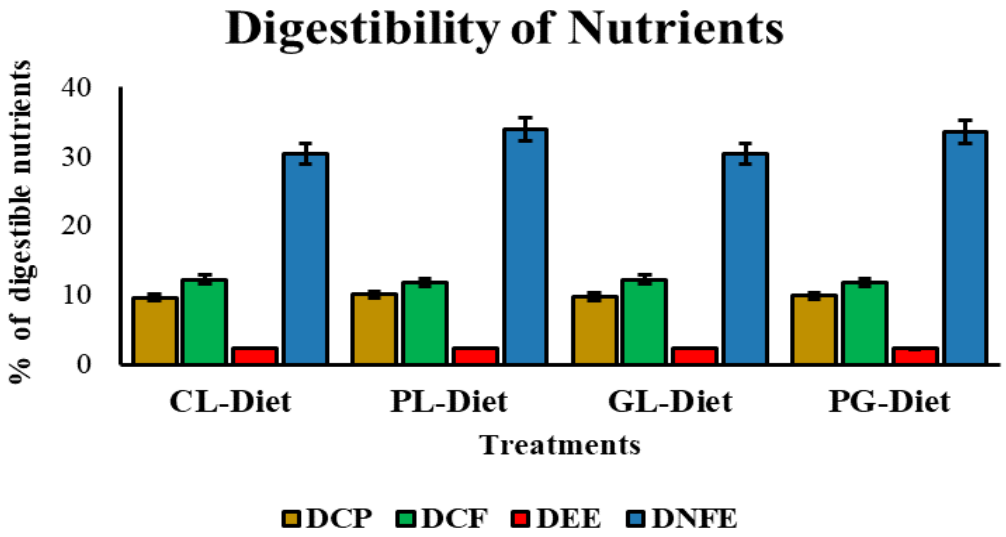

Figure 1: Effect of herbal supplementation on nutrients digestibility (g/100g DM) of sheep

DCP: *Digestible Crude protein;* ***DCF:*** *Digestible crude fibre;* ***DEE:*** *Digestible ether extract;* ***DNFE:*** *Digestible Nitrogen Free Extract;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm);* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM Plantain and 5g DM garlic leaves supplementation.*

TDN digestibility

Supplementation of different herbs with TMR pellet found to have significant effect on total digestible nutrients (TDN). TDN was significantly higher (P < 0.05) in plantain supplementing group, then chronologically showed the following consequence plantain and garlic leaf mixed herbs supplemented group, garlic leaf supplemented group compared to control group (Figure 2).

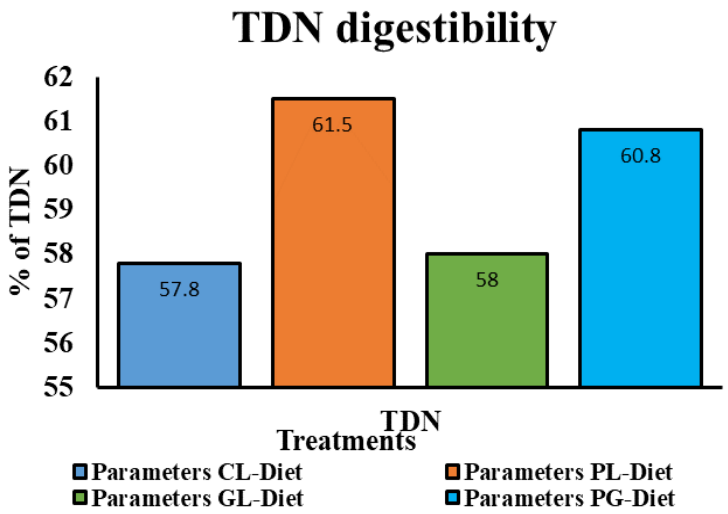

Figure 2: Effect of herbal supplementation on TDN digestibility (g/100g DM) of sheep
TDN: *Total Digestible Nutrients;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM Plantain and 5g DM garlic leaves supplementation.*

Ahmed *et al*. (2009) reported that appropriate quantities of plantain and neem leaves stimulated the appetite and digestion process of calves. Afshar *et al*. (2012) also reported that higher nutrient digestibility was found in garlic leaf supplemented sheep compared to control. In this experiment, higher DNFE and TDN in all herbal supplementation groups compared to control group showed. This result is in agreement with the findings of Shuvo *et al*. (2018) who found higher TDN in sheep by using 5% plantain supplementation in TMR diet and Busquet *et al*. (2005) also found similar result in native sheep by using 1% garlic leaf supplementation. One of the probable cause for getting higher nutrient digestibility in herbal supplemented group was microbial modulation in the rumen due to action of bioactive components that are present in herbs. This hypothesis was supported by Hadiya *et al*. (2009) who reported that medicinal plants had anti-microbial characteristics could affect the inappropriate microbes in the rumen and reduced unnecessary nutrient loss. More specifically, Plantain contains aucubin, aectiosides, catalpol (Al-Mamun *et al*., 2007) that reduce activities of methanogenic bacteria by lowering H_2 sink in the rumen ((Meyer *et al*., 2006). As a result, lower nutrients were lost in plantain supplemented group (PL-diet) and attributed higher TDN. Like plantain, garlic leaves also contain bioactive components allicin, diallyl disulfide (Chung *et al*., 2006) which have inhibitory activities on methanogenic bacteria (Ikyume *et al*., 2005). Busquet *et al*. (2005b) reported that lower molar proportion of acetate and higher proportions of propionate and butyrate was found in sheep supplemented with garlic leaves and this finding also justify that, due to reducing methanogenic bacteria more H_2 ion would be used for the production of propionic acid which promoted lower nutrient loss in garlic supplemented group (GL-diet).

Growth performance

A significant difference (P > 0.05) in body weight gain, dry matter intake, CP intake and feed conversion ratio (Figure 3) between all dietary treatment groups on aligned. Treatment groups however, exhibited higher daily weight gain compared to control. Daily DM and CP intake was

highest for PL-diet group which was similar to PG-diet group compared to other treatment groups. Lowest FCR was found in PL-diet group compared to other treatment groups.

Live weight gain was found higher for lambs grazing plantain than ryegrass swards when both were offered an allowance of the diet (Moorhead *et al*., 2002) which support the result of the present experiment as higher daily live weight gain in plantain supplemented group (PL-diet) compared to other groups. Fraser *et al*. (1988) and Rumball *et al*. (1997) also reported higher growth rates and lower feed conversion ratio of calves and lambs grazing mixed swards containing plantain herb. Bampidis *et al*. 2005 reported that higher daily weight gain of growing lamb was found in garlic leaves supplemented group compared to normal basal diet that also justify our findings. Supplementation of different herbs improve DM intake due to modulated microbial activities in rumen (Feldberg *et al*., 1988) as well as action of different bioactive components in arcuate nucleus and the para ventricular nucleus (Crespo *et al*., 2014). Yang *et al*. (2007) reported higher DM intake in sheep by using garlic leaf supplementation and similar findings was reported by Shuvo *et al*. (2018) with plantain herbs. Improved growth rates in all herbal supplemented groups might be due to higher nutrient turnover, lower nutrient loss and lower parasitic count. Fulkerson *et al*. (2008) stated that improvement of lamb's growth rate from grazing plantain might be due to increased metabolizable protein supply which was also true for garlic leaf supplemented group (Ferme *et al.* 2004). Al-Mamun *et al*. (2008) reported that plantain helped to increase whole body protein synthesis in Sheep and Cardozo *et al*. (2004) reported that garlic leaf reduced ammonia N and increased peptide and amino acid N concentrations, suggesting that deamination were inhibited. Bioactive components present in both plantain and garlic leaves are polyphonic in nature (Al-Mamun *et al*., 2007) and having a positive impact on energy metabolism by reducing methanogenic population in sheep (Ma *et al*., 2015).

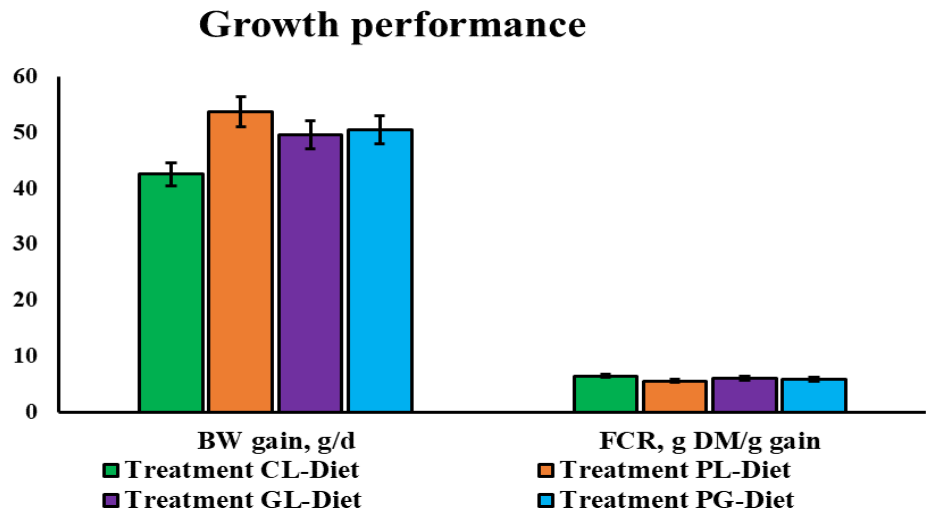

Figure 3: Effect of herbal supplementation on growth performance of sheep
FCR: *feed conversion ratio;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM Plantain and 5g DM garlic leaves supplementation*

11.2. Determination of the level of antioxidant and cholesterol in the meat from experimental sheep:

Plasma metabolites

Supplementation of different herbs with TMR pellet had significant effect (P<0.05) on plasma glucose, blood urea nitrogen (BUN), triglyceride and low density lipoprotein concentration (LDL-cholesterol) in sheep (Figure 4). Higher plasma glucose and triglycerides was found in control group (CL-diet) and garlic leaf supplemented group (GL-diet) compared to other groups. BUN was higher in plantain supplemented group (PL-diet) which was similar to mixed herbs supplemented group (PG-diet) than control and garlic supplemented group. Lowest plasma total cholesterol and LDL-cholesterol was found in plantain and garlic supplemented group compared to control and mixed herbs supplemented group.

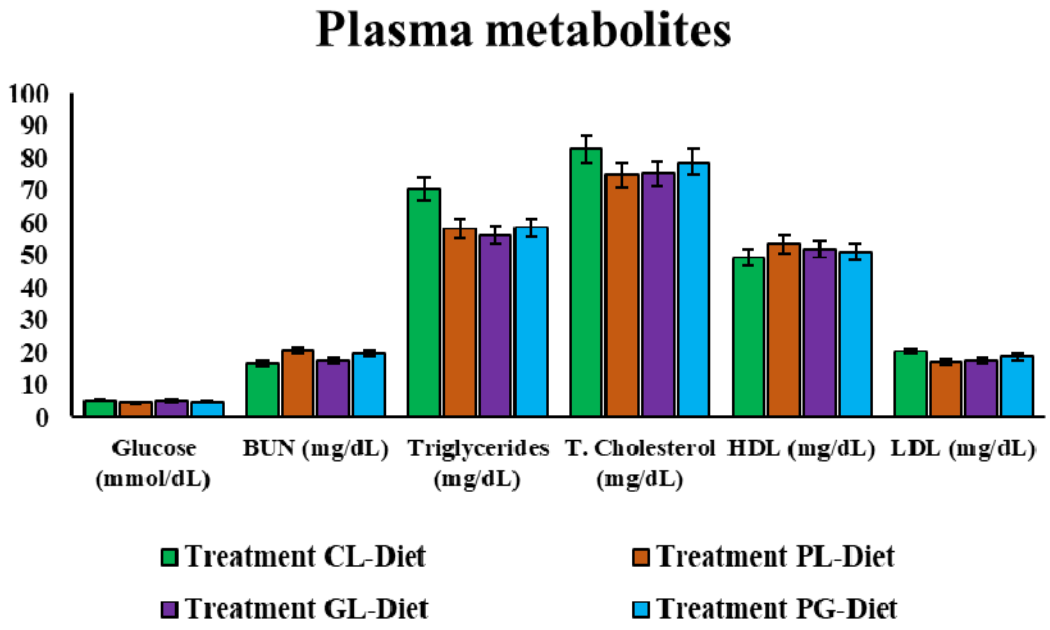

Figure 4: Effect of herbal supplementation on plasma metabolites in sheep

BUN: *blood urea nitrogen;* ***T. Cholesterol****: total cholesterol;* ***HDL:*** *high density lipoprotein;* ***LDL:*** *low density lipoprotein;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaves supplementation.*

Yoshida *et al*., (2013) reported that supplementation of plantain reduced serum free-fatty acid and glucose levels in sheep which supports the present finding of lower plasma glucose level in both plantain supplementation group (PL-diet) and mixed herb supplementation group (PG-diet). Plantain helps to boost up protein turnover rate in sheep (Al-Mamun *et al*., 2008) and this would be the reason of raising blood urea level in both plantain and mixed herbs supplemented group. This result can also be justified by findings of Yang *et al*. (2007) who reported that garlic leaf supplementation had no effect on ruminal concentration of NH_3-N. Concentration of total cholesterol, HDL-cholesterol and LDL- cholesterol in blood are largely depends on the polyunsaturated fatty acid content of the feed. Lipid profile of plasma indicates the physiological condition and nutritional status of the animal. Yamabe *et al*. (2010) reported that plantain (*Plantago lanceolata* L.) contains iridoid

glycosides that exert prevention and improvement on abnormal glucolipid metabolism by reducing oxidative stress and formation of advanced glycation end products that exhibited lower TGs, total cholesterol level in treatment group which justify our finding for plantain and mixed herb supplemented group. Ali *et al*. (2010) reported that allicin that was extracted from garlic leaves that reduced the availability of 3-Hydroxy-methyl-glutaryl concentration in rat that might be the probable reason for lowering serum cholesterol concentration. Combined effect of allicin from garlic leaf and aucubin from plantain through supplementation of mixed herbs to the sheep might be attributed for lowering serum TGs, total cholesterol and LDL-concentration.

Blood profile and immunity

Herbal supplementation with TMR pellet had significant effect ($P < 0.05$) on neutrophil & lymphocyte count (Table 3). Highest value (1.82 x 10^9/L for neutrophil and 5.02 x 10^9/L for lymphocytes) was found for plantain supplemented group (PL-diet) compared to other dietary groups. Herbal supplementation also exhibited positive impact to raise serum albumin and globulin concentration (Figure 5). Higher concentration of immunoglobulin G1, immunoglobulin G2, immunoglobulin M, immunoglobulin A were found in PL-diet group than mixed herbs supplemented group (PG-diet) and garlic leaf supplemented group (GL-diet), and lowest value was found in control group (CL-diet).

This research has shown a positive relationship between plantain supplementation and the immunoglobulin concentration. Christaki *et al*. (2004) reported that Chinese herbal crude extracts from Lentinus edodes (8.0% purity), Tremella fuciformis (10.0% purity), or Astragalus membranaceus (31.1% purity) at a concentration of 1 g kg−1, significantly increased the levels of cecal-specific IgA and IgG, and antigen-specific proliferation of splenocytes in young chickens. Sang-Oh *et al*. (2010) reported that, using Cinnamon (Cinnamomi cassiae cortex) a medicinal herb in broiler diet increase blood immunoglobulin levels due to greater proliferation of cells in immune organs compared to the control group. Wang *et al*. (2016) cited that Chinese herb mixture had positive impact on immunity status in sheep. All of these findings are in agreement with our present result.

Table 3: Effect of herbal supplementation on blood profile and immunity in Sheep

Parameters	**Treatment**			
	CL-Diet	**PL-Diet**	**GL-Diet**	**PG-Diet**
Blood profile				
RBC (×10^{12}/L)	11.30	11.27	11.28	11.23
WBC (×10^9/L)	6.52	6.95	6.53	6.77
Neutrophil (×10^9/L)	1.66	1.82	1.66	1.73
Lymphocyte (×10^9/L)	4.54	5.02	4.63	4.81
Monocyte (×10^9/L)	0.28	0.27	0.28	0.27
Eosinophil (×10^9/L)	0.28	0.30	0.27	0.30
Hb (g/L)	118.5	121.1	119.9	118.7

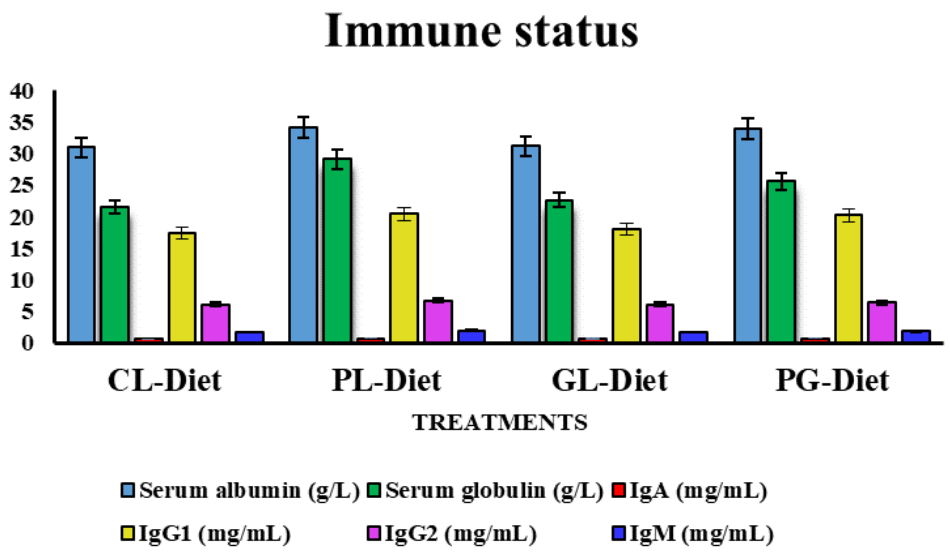

Figure 5: Effect of herbal supplementation on immunity in Sheep

RBC: *red blood cell;* ***WBC:*** *white blood cell;* ***IgA:*** *immunoglobulin A;* ***IgG1:*** *immunoglobulin G1;* ***IgG2:*** *immunoglobulin G2;* ***IgM:*** *immunoglobulin M;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaves supplementation.*

The immunity status in the animal body is linked reciprocally to oxidative stress and the ingestion of antioxidants, because free radicals play important roles in immunity and signal transduction (Miyazaki *et al*., 2001). The excessive free radicals generated by an animal body in an abnormal state could attack polyunsaturated fatty acid and cause lipid peroxidation in the cell membrane (Turner *et al*., 2004). As both plantain (Al-Mamun *et al*., 2007) and garlic leaf (Chung *et al*., 2006) has higher potentiality to scavenge free radical in animal body that might be possible mechanism to raise serum immunoglobulin concentration in herbal supplemented group.

Hormone level and enzymatic activities

Herbal supplementation with TMR pellet found to have no significant effect ($P < 0.05$) on growth hormone (GH), insulin, glucagon and thyroid stimulating hormone level (TSH) in sheep. But, a positive tendency was found between herbal supplemented groups. Lowest alanine transaminase (ALT) and creatine kinase activities were found in plantain supplemented group (PL-diet) compared to other treatment group (Table 4). But there was no significant difference ($P > 0.05$) in aspartate transaminase (AST) and alkaline phosphatase level among the treatment groups.

Table 4: Effect of herbal supplementation on hormone level and enzymatic activates in sheep

Parameters	Treatment				SEM	*P*-value
	CL-Diet	PL-Diet	GL-Diet	PG-Diet		
Hormone Level						
Growth hormone (ng/mL)	0.38	0.81	0.56	0.69	0.18	0.09
Insulin (pIU/mL)	11.97	19.11	13.76	16.67	1.91	0.07
Glucagon (pg/mL)	141.6	136.0	139.3	138.4	3.9	0.47
TSH (pIU/mL)	0.21	0.28	0.23	0.27	0.02	0.11
Enzymatic Activities						
ALT (U/L)	20.19	14.56	21.72	19.77	1.01	<0.001
AST (U/L)	43.12	40.50	42.13	41.71	1.02	0.11
Creatine kinase (U/L)	150.3	118.5	137.2	133.7	7.7	0.04
Alkaline phosphatase (U/L)	185.4	194.1	197.1	181.3	15.1	0.78

TSH: *thyroid-stimulating hormone;* ***ALT:*** *alanine transaminase****; AST:*** *aspartate transaminase****; CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaf supplementation.*

Liu *et al*. (2007) reported that herbal supplementation tended to raise the plasma GH level in sheep. But in the present experiment, there was no significant difference between treatment groups in terms of hormonal level. However, a positive tendency was found between herbal supplemented groups specially plantain supplemented group (PL-diet). This result was in agreement with the finding of Wang *et al*. (2016) with Chinese mixed herbs. Although the plasma hormonal level tended to rise, the herbal supplementation failed to regulate the hypothalamic-pituitary-thyroid axis. The interactions among three endocrine glands, such as the hypothalamus, pituitary gland and adrenal gland, constitute the hypothalamic-pituitary-adrenal axis (HPA axis), a major part of the neuroendocrine system, which controls reactions to stress and regulates many body processes (Kim *et al*., 2013). Experimental studies have investigated many types of stress, and their effects on the HPA axis in many circumstances (Ranabir & Reetu, 2011). Insulin is central to regulating carbohydrate and fat metabolism in the body, which lowers blood glucose level (Prosser, 1991). Its effect is opposite to that of glucagon. Lowest plasma insulin level was found in plantain supplemented group than mixed herbs group, showing that plantain might reduce the blood glucose level which was supported by Sano *et al*. (1998) who cited that tissue responsiveness to insulin was enhanced by the plantain diet in sheep.

Enzymatic activity in the blood is linked strongly to the functional status of corresponding tissues that secrete enzymes (Prosser *et al*., 1991). Generally, enzymes exist in the blood with relatively constant activity, but this activity can fluctuate with certain changes suffered by the related tissue. Plasma ALT activity is most commonly associated with the liver, and is often measured clinically as part of a diagnostic evaluation of hepatocellular injury to determine liver health (Ghouri *et al*., 2010). Plasma ALT activity declined significantly due to supplementation of plantain (PL-diet), suggesting that this herb might protect the liver of sheep from damage. Creatine kinase (CK) catalyses the conversion of creatine and uses adenosine triphosphate (ATP) to generate phosphocreatine (PCr) and adenosine di-phosphate (ADP). This CK enzyme reaction is invertible and thus ATP can be created from PCr and ADP. In tissues and cells that consume ATP rapidly, especially skeletal muscle, PCr acts as energy reservoir for the rapid buffering and revivification of ATP, as well as for

intracellular energy transport by the PCr shuttle or circuit (Hekimsoy & Oktem, 2005). The pronounced reduction of plasma CK activity due to supplementation of plantain herb illustrated that this herb might benefit the energy supply of muscle as well as prevents cacotrophy in sheep compared to garlic leaf and control group.

Serum free radicle scavenging capacity

Supplementation of different herbs with TMR pellet had significant effect ($P < 0.05$) on serum total antioxidant capacity (TAC), Superoxide dismutase (SOD), Glutationine peroxidase (GPx) and Catalase level on sheep (Figure 6 and 7). Highest serum free radicle scavenging capacity was found in plantain supplemented group (PL-diet) and then mixed herbs supplemented group (PG-diet) and garlic leaf (GL-diet) compared to control group.

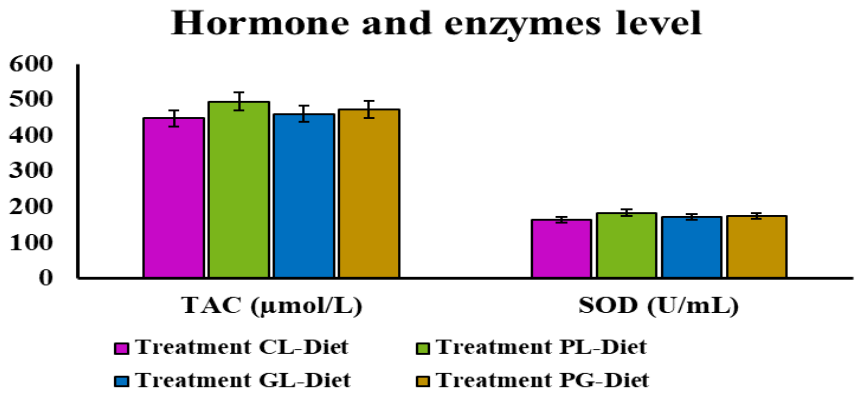

Figure 6: Effect of herbal supplementation on hormone level and enzymatic activates (TAC and SOD) in sheep

TAC: *Total antioxidant capacity;* ***SOD:*** *Superoxide dismutase;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM Plantain and 5g DM garlic leaves supplementation.*

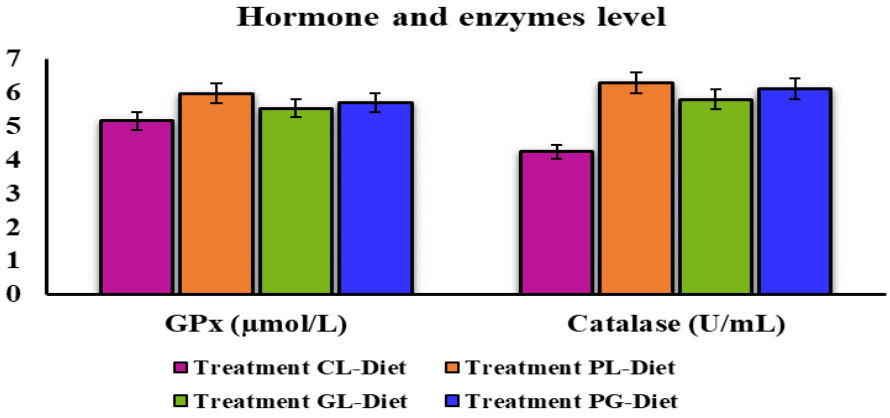

Figure 7: Effect of herbal supplementation on hormone level and enzymatic activates (GPx and Catalase) in sheep

GPx*: Glutathione peroxidase;* ***CL-diet:*** *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM Plantain and 5g DM garlic leaves supplementation.*

The immunity status in the animal body is linked reciprocally to oxidative stress and the ingestion of antioxidants, because free radicals play important roles in immunity and signal transduction (Miyazaki *et al*., 2001). The excessive free radicals generated by an animal body in an abnormal state could attack polyunsaturated fatty acid and cause lipid peroxidation in the cell membrane (Turner *et al*., 2004). There is a balance between the production and removal of free radicals in the body. The overproduction of free radicals could be eliminated by anti-oxidative enzymes including GPx, SOD and catalase. In this experiment, total anti-oxidant capacity (TAC) was higher in all herbal treatment group compared to control group. Highest value was found in plantain supplemented group (PL-diet) that might be due to presence of bioactive components aucubin, catalpol and acteoside. This hypothesis could be justified by the finding of Al-Mamun *et al*. (2007) who reported that bioactive components present in plantain had higher free radical scavenging activities. And this free radical might be attributed to raise the total antioxidant capacity in PL-diet and PG-diet group. On the other hand, garlic compounds such as alliin, ally' cysteine and ally' disulfide scavenge hydroxyl radicals through thiol exchange mechanism (Chung *et al*., 2006) that might be attributed for higher TAC, SOD, GPx, catalase level compared to control group.

Carcass characteristics and fat deposition

There was no significant difference ($P < 0.05$) in carcass characteristics among the treatment groups rather than caul fat, pelvic fat and dressing percentage (Table 5). Caul fat and pelvic fat was lowest in plantain supplemented group (PL-diet) than mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet). Highest dressing percentage was found in PL-diet group than the other treatment groups.

Table 5: Effect of herbal supplementation on carcass characteristics and fat deposition in sheep

Parameters	Treatment				SEM	*P*-value
	CL-Diet	PL-Diet	GL-Diet	PG-Diet		
Hot carcass wt.(kg)	5.2	5.8	5.5	5.3	0.28	0.240
Fore limb (%)	0.91	0.84	0.85	0.87	0.11	0.525
Hind limb (%)	0.87	0.79	0.81	0.77	0.26	0.119
Neck (%)	2.93	3.81	3.19	3.46	0.27	0.087
Fore leg (%)	4.07	3.76	4.16	3.89	0.03	0.145
Hind leg (%)	4.09	4.78	4.54	5.65	0.05	0.117
Caul fat (%)	1.33	0.91	1.01	0.97	0.09	0.036
Pelvic fat (%)	0.69	0.30	0.51	0.47	1.04	<0.001
Dressing percentage (%)	40.5	44.2	41.5	43.6	0.08	<0.001

CL-diet: *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM Plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaves supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaves supplementation.*

Plantain and garlic leaf are not only a source of bioactive components but also a good source of proteins and minerals which causes higher protein retention and availability leading to better performance and yielded carcass in sheep (Al- Mamun *et al*. 2008, Martins *et al*., 2016). Plantain reduces abdominal fat accumulation, plasma non-esterified fatty acids content in sheep (Al-Mamun *et al*., 2010) which support the result of the present finding. Acteoside that are present in plantain (Al-Mamun *et al*., 2007b) stimulates leptin expression and cholesterol catabolism, suppressing fatty acid synthesis, and accelerating fatty acid β-oxidation (Yang et *al.,* 2015) and this is one of the reason for getting lower caul fat and pelvic fat in PL-diet and PG-diet group. Gebhardt and Beck (1996); Cho

and Xu (2000) reported that inhibitory effects of garlic organosulfur compounds on cholesterol biosynthesis in the hepatocytes by inhibition of the HMG-CoA reductase might be the probable reason for lower pelvic and caul fat in sheep.

Mutton quality

There was no significant difference in proximate components of mutton except ether extract (EE) content among the dietary treatment groups. EE content was significantly (P<0.05) lower in all herbal supplementation group compared to control group (Table 6). Dripped loss and cooking loss had no significant defference but higher rib eye area was exhabited higher in plnatin supplemented group (PL-diet) than mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet). Lower mutton cholesterol was found in PL-diet group compared to other treatment groups (Figure 8).

Table 6: Effect of herbal supplementation on proximate composition and cholesterol in mutton

Parameters	Treatment				SEM	*P*-value
	CL-Diet	PL-Diet	GL-Diet	PG-Diet		
Moisture (%)	72.63	72.52	72.64	72.62	0.06	0.08
Crude protein (%)	21.37	21.89	21.61	21.80	0.06	0.07
Ether extract (%)	4.55	3.99	4.08	4.17	0.07	0.001
Ash (%)	1.10	1.07	1.12	1.09	0.01	0.56
Nitrogen free extract (%)	72.98	73.04	73.19	72.94	0.04	0.07
Mutton cholesterol	111.2	93.7	101.21	98.23	0.09	0.04

CL-diet: *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaf supplementation.*

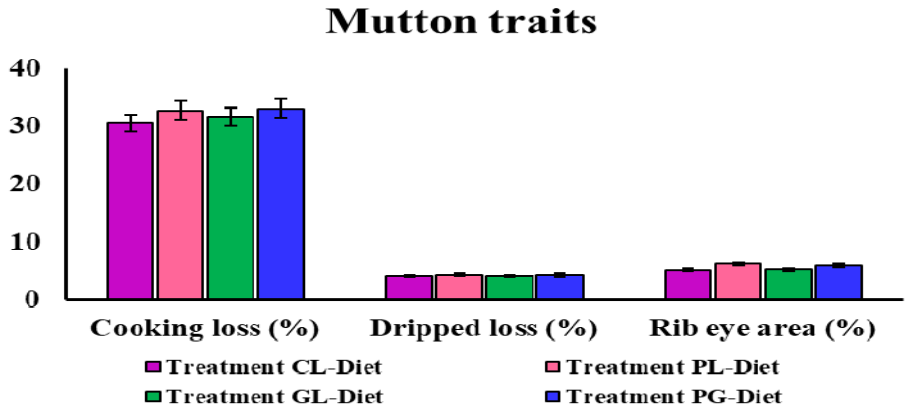

Figure 8: Effect of herbal supplementation on meat traits in sheep

CL-diet: *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5g DM garlic leaf supplementation.*

In a previous experiment, Shuvo *et al*. (2018) found lower fat in mutton by using 5% plantain herb supplementation in total mixed ration based feeding system. Similar findings were demonstrated by using 0.7 % Chinese medicinal herbs mix (Tu-Fa *et al*., 2013) and 1% garlic leaf (Gebhardt *et al*., 1996) supplementation. All of these findings justify our result that was lower mutton fat and cholesterol in herbal supplemented group compared to control.

Most probably bioactive components in the herbs might have inhibitory effect on fat and cholesterol biosynthesis. This hypothesis was supported by the findings of Gebhardt *et al*. (1996) and Cho *et al*. (2000), which had demonstrated that inhibitory effects of garlic organosulfur compounds on cholesterol biosynthesis in the hepatocytes by inhibition of the HMG-CoA reductase resulting lower mutton fat and cholesterol. Acteoside, however present in plantain (Al-Mamun *et al*., 2007b) stimulates leptin expression and cholesterol catabolism, suppressing fatty acid synthesis, and accelerating fatty acid β-oxidation (Yang *et al*., 2015) and this is one of the reason for getting lower mutton fat and cholesterol in PL-diet and PG-diet group. Highest rib eye area was found in PL-diet group which was also similar to the finding of Shuvo *et al*. (2018). Plantain however increases whole body protein synthesis (Al-Mamun *et al*., 2008) that might be attributed for higher rib eye area in PL-diet and PG-diet group.

Mutton fatty acid

There was a significant difference in saturated fatty acid (SFA) and unsaturated fatty acid (USFA) among the dietary treatment groups. SFA content was significantly (P<0.05) lower in all herbal supplementation group compared to control group (Table 7 and Figure 9) and vice versa for USFA. Monounsaturated fatty acid (MUFA) was lower (P<0.05) in all herbal supplemented group compared to control. Linoleic acid, major interest for producing functional meat was highest in plantain supplemented group (PL-diet) than mixed herbs group (PG-diet), garlic supplemented group (GL-diet) and control group (CL-diet).

Kasapidou *et al*. (2001) stated that higher tissue concentrations of antioxidant are associated with higher amounts of both n-6 and n-3 PUFA in tissues. In this experiment higher serum antioxidant was found in all herbal supplemented groups (Figure 6 and 7) and consequently got higher PUFA in treatment group. Both plantain (Al-Mamun *et al*., 2007) and garlic leaf (Chung *et al*., 2006) are rich source of antioxidant that might be attributed for getting higher PUFA in PL-diet, GL-diet and PG-diet group.

Table 7: Effect of herbal supplementation on mutton fatty acid

Parameters	Treatment				SEM	*P*-value
	CL-Diet	PL-Diet	GL-Diet	PG-Diet		
Saturated Fatty Acids %	**50.75±0.81**	**49.94±0.62**	**49.98±0.31**	**49.82±0.41**	0.14	<0.001
Myristic Acid (C14:0)	1.85±0.01	2.10±0.03	1.46±0.01	1.86±0.01	0.07	<0.001
Palmitic Acid (C16:0)	23.68±0.51	22.87±0.21	23.13±0.37	23.97±0.12	0.16	<0.001
Margaric Acid (C17:0)	1.84±0.02	2.12±0.02	2.02±0.01	1.63±0.06	0.22	<0.001
Stearic Acid (C18:0)	23.38±0.33	22.9±0.41	23.37±0.17	23.37±0.74	0.47	0.040
Unsaturated Fatty Acids %	49.25±0.01	50.06±0.02	50.02±0.03	50.18±0.04	0.17	<0.001
i) Monounsaturated Fatty Acids %	42.15±0.09	40.81±0.07	41.6±0.13	41.7±0.11	0.37	0.030
Myristoleic Acid (C14:1)	0.55±0.03	0.46±0.05	0.56±0.07	0.48±0.09	0.02	<0.001
Palmitoleic Acid (C16:1)	0.16±0.01	0.30±0.04	0.11±0.25	0.15±0.02	0.31	<0.001
Oleic Acid (C18:1)	41.43±1.89	40.05±2.13	40.93±1.77	40.07±2.10	0.27	0.120
ii) Polyunsaturated Fatty Acids %	7.10±0.13	9.21±0.04	8.42±0.09	8.48±0.07	0.39	0.010
Linoleic Acid (C18:2)	3.99±0.31	5.28±0.67	4.98±0.59	5.09±0.51	0.21	<0.001
Linolenic Acid (C18:3)	1.18±0.09	1.02±0.07	1.15±0.03	1.09±0.04	0.03	<0.001
Arachidonic Acid (C20:4)	1.92±0.13	2.91±0.27	2.31±0.21	2.45±0.32	0.23	<0.001

CL-diet: *Road side grass, rice straw, wheat bran, mustard oil cake, molasses, common salt based total mixed ration pellet (6 mm),* ***PL-diet:*** *CL-diet + 10g DM plantain supplementation;* ***GL-diet:*** *CL-diet + 10g DM garlic leaf supplementation;* ***PG-diet:*** *CL-diet + 5g DM plantain and 5gD M garlic leaves supplementation.*

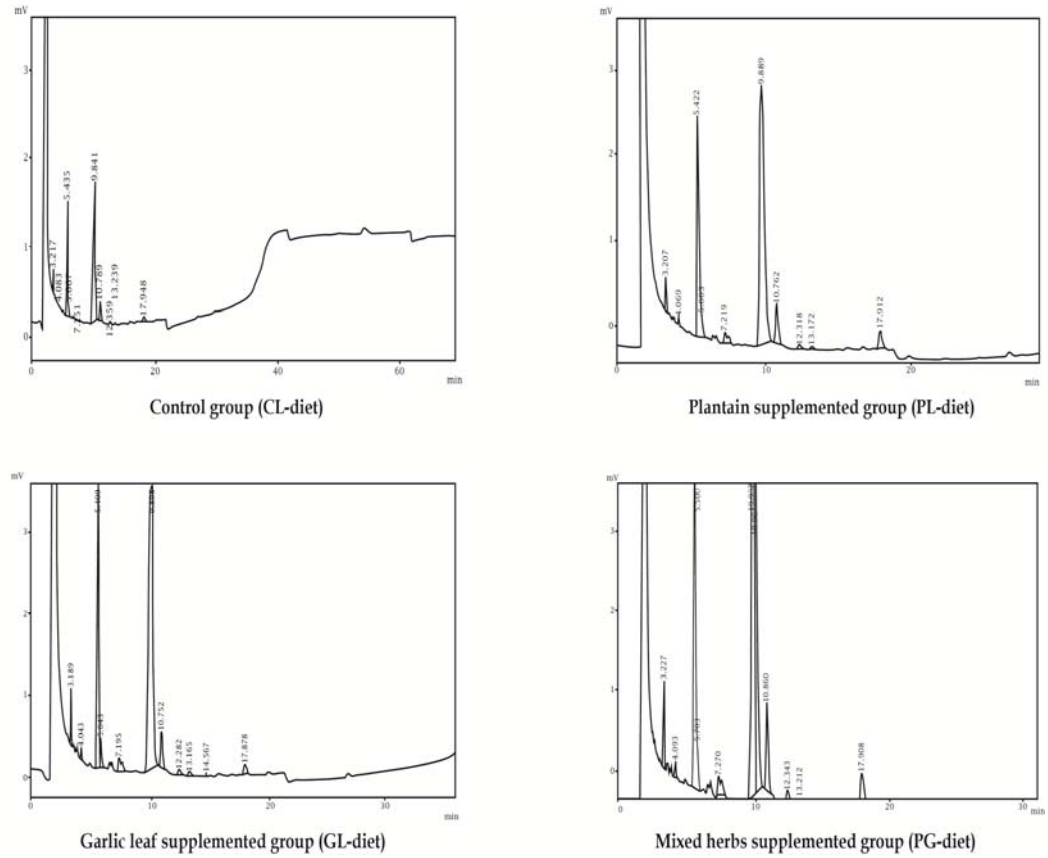

Figure 9: Mutton fatty acid chromatograph of different treatment groups

12. Research Highlights/findings:

i. Herbal supplementation with TMR diet improved body weight gain, the digestibility of diet and dressing percentage of sheep.

ii. It revealed that Plantain diet (PL-diet) reduced the caul and pelvic fat content compared to control group.

iii. Plantain diet (PL-diet) and then mixture of plantain and garlic diet (PG-diet) not only boosted up serum antioxidant status (TAC, SOD, GPx, and Catalase) but also serum immunoglobulin (IgG, IgM, IgA) concentration.

iv. PL-diet, then PG-diet reduced blood and mutton total cholesterol content.

v. PG-diet increased the mutton linoleic acid content and second higher mutton fatty acid content was found in PL-diet.

vi. Plantain-diet and mixture of plantain and garlic diet (PG-diet) might be used for the production of healthy, lean and functional mutton in Bangladesh context for the betterment of rural farmers.

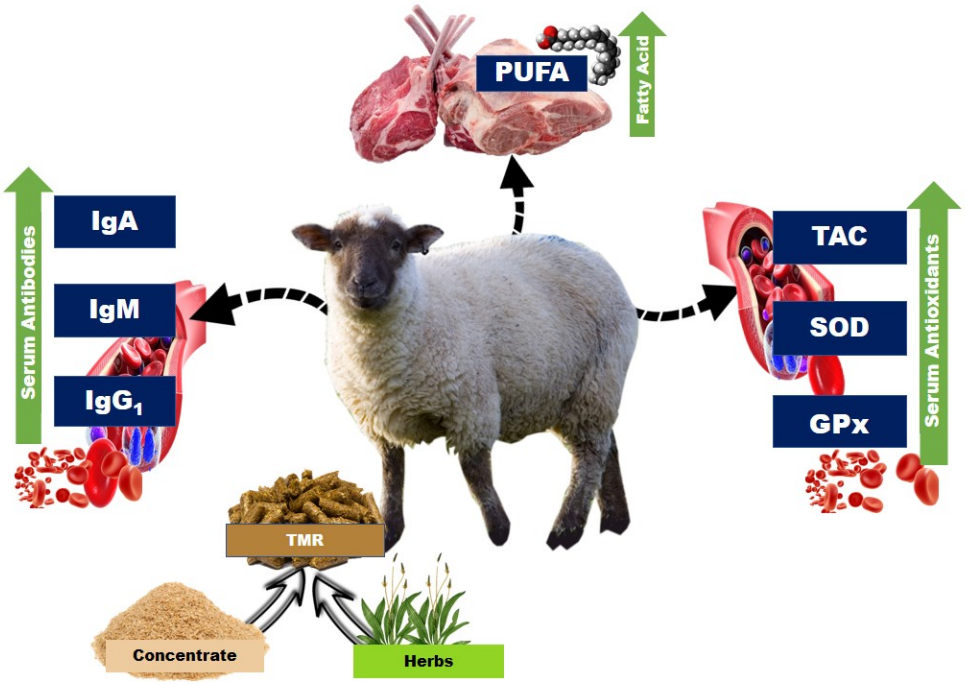

Figure 10: Effect of herbal supplementation on physiochemical changes of sheep

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP target		Achievement		Remarks
	Phy (#)	Fin (TK)	Phy (#)	Fin (TK)	
(a) Office equipment	Laptop, scanner, printer, executive chair, executive table and file cabinet	150000	Laptop, scanner, printer, executive chair, executive table, file cabinet	149700	Timely procured
(b) Lab & Field equipment			.		
(c) Other capital items					

2. Establishment/renovation facilities: Not applicable

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

3. Training/study tour/seminar/workshop/conference organized: Not applicable

Description	Number of participant			Duration (Days/weeks/months)		Remarks
	Male	Female	Total			
(a) Training						
(b) Workshop						

C. Financial and Physical progress

Fig in TK.

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual Staff Salary	608972	487825	513627	-25802	84%	Fund not yet released
B. Field Research / Lab expenses and supplies	3200000	3198225	3198225	0.0	100%	
C. Operating Expenses	175000	164250	174250	-10000	100%	Fund not yet released
D. Vehicle Hire and Fuel, Oil & Maintenance	235000	185000	235000	-50000	100%	Fund not yet released
E. Training/Workshop/ Seminar, etc.	0	0	0	0.0		
F. Publications and printing	165000	65000	65000	0.0	39 %	Fund not yet released
G. Miscellaneous	40000	40000	40000	0.0	100%	
H. Capital Expenses	150000	149700	149700	0.0	100%	

D. Achievement of sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objective	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To test the efficacy of medicinal herbs on the growth performances of sheep	➢ Herb selection through review of literature. ➢ Land selection and preparation for herb cultivation. ➢ Cultivation of Plantain (*Plantago lanceolata* L.) and garlic leaves (*Allium sativum*). ➢ Feeding trial using 32 sheep to know the efficacy of these two herbs and their mixture on sheep ➢ Study of growth performances of experimental sheep.	Plantain diet (PL-diet) and Plantain-garlic (PG) diet was found to be significantly better than control diet. Plantain diet and plantain-garlic diet significantly improved live weight gain through increasing nutrients and TDN digestibility.	A feeding strategy using plantain diet and plantain-garlic diet might be developed.
To identify the antioxidant and cholesterol level of mutton	➢ Plasma metabolites of experimental sheep were determined. ➢ Immune status and hormonal level of experimental sheep were performed. ➢ Serum antioxidant level of experimental sheep was determined. ➢ Cholesterol level of serum and meat were determined. ➢ Carcass characteristics of experimental sheep were observed. ➢ Finally fatty acid level of experimental sheep was estimated.	These diets improved plasma intermediate nutrients and immunity through increasing the level of antioxidants and enzymes activity in sheep. Mutton cholesterol was found numerically better in plantain diet and mutton fatty acid level was significantly higher in Garlic diet.	A suitable feeding strategy using plantain herbs and garlic-plantain mixture with TMR might be used for the production of healthy, safe and antioxidant rich mutton in Bangladesh context.

E. Materials Development/Publication made under sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/booklet/leaflet/flyer etc.		2500 peices leaflet	
Journal publication			
Information development			
Other publications, if any		One MS thesis	Production of low cholesterol healthy mutton by using natural herbs

F. Technology/Knowledge generation/policy support (as applied):

i. Generation of technology (commodity & Non-commodity):

None

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

Plantain diet (PL-diet) is better than Plantain-garlic diet (PG-diet) and then Garlic diet (GL-diet) for the production of safe and healthy mutton production.

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

Plantain diet (PL-diet) and combination of plantain and garlic diet (PG-diet) might be used for sustainable development of sheep in Bangladesh context.

iv. Policy support

Livestock policy makers should consider making a suitable feeding strategy using plantain herbs and garlic-plantain mixture with TMR for the production of healthy, safe and antioxidant rich mutton in Bangladesh context.

G. Information regarding Desk and Field Monitoring

i) Desk monitoring [description & output of consultation monitoring workshops/ seminars etc.]:

ii) Field monitoring [time & No. of visit, tear visit and output]:

The project was completed according to the work-plan and the monitors were observed the project March 2, 2018 and satisfied without any claims.

H. Lesson Learned (if any)

I. Challenges (if any)

i. The rural people or women are not aware of the advantages of sheep farming with scientific feeding approaches.

ii. A sheep and mutton marketing channel are not properly developed in our country.

iii. It`s very difficult for the common people to understand the low cholesterol and value added mutton. Therefore, attempt should be taken to make awareness among common people about the health benefits of antioxidant rich, low cholesterol, lean and safe mutton.

Signature of the Principal Investigator
Date ……..
Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal