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Effect of supplementation of neem (Azadirachta indica) leaves to lactating cows for improving milk production in traditional farming system

M Moniruzzaman[1], MAS Khan[1], M Akter[2], Ashikur Rahman[3], Abdur Rahman[3], M Asaduzzaman[3]*

[1]Dept. of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Dept. of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
[3]Dept. of Dairy Science, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

* Correspondence: asad.dasc@sau.edu.bd

ABSTRACT

The present experiment was carried out to evaluate the efficacy of neem leaves on the performance of early lactating cows feeding with straw-based traditional diet (rice straw, wheat straw and roadside grass etc.). For this purpose, six lactating cows were selected based on early lactation and severity of infection in terms of gastrointestinal nematode. In each cow, neem leaves were supplemented at the rate of 200 mg/kg live weight of the animal along with the straw-based traditional diet. The selected animals were observed and data were collected 4 weeks before and 4 weeks after treating with neem leaves. Feces were collected from the cows weekly for the observation of parasites. From the findings, it was observed that the average egg per gram of feces (EPG) was significantly ($p<0.05$) lowered after supplemented with neem leaves. The average body weight and milk production of the lactating cows were significantly increased after treatment. The results indicate that supplementation of neem leaves had a positive effect on the performance of lactating cows and it can be used for better performance of lactating cows under village conditions where cows are reared on straw-based traditional diet.

Keywords: Neem leaves, supplemented feed, parasites, body weight and milk production,

INTRODUCTION

The environmental conditions of Bangladesh are very conducive to parasitic infection in domestic animals (Kabir *et al*., 2011). The high average rainfall (100 mm), relative humidity (80%) and temperature (26°C), together with stagnant water in low-lying areas of Bangladesh, improper husbandry practices and poor nutritional status of animals favor greatly the incidence of gastrointestinal nematodiasis in cattle (Tulu and Lelisa, 2016). Gastrointestinal nematode parasitism of ruminant causes significant loss in the productivity of animals in developing countries through mortality and reduced production of milk, meat and work potential (Fthenakis and Papadopoulos, 2018; Lashari and Tasawar, 2011). Chemical anthelmintics have long been considered as the only effective way of controlling parasitic infection (Enejoh and Suleiman, 2017). However, as these are very expensive and unavailable to farmers in rural areas of Bangladesh, livestock producers are not interested to use these anthelmintics. Furthermore, regular use of chemical anthelmintics develops resistance of helminths to various anthelmintics compounds (Wolstenholme *et al*., 2004). For these reasons, interest in screening medicinal plants for anthelmintic activity remains of great scientific interest despite the extensive use of synthetic chemicals in modern clinical practices all over the world.

Gastrointestinal parasitism is one of the major problems affecting the productive performance of animals in Bangladesh (Akanda *et al*., 2014). The use of herbal remedies is cheap, locally available and convenient to administer. Regular use of effective herbal anthelmintics in livestock ration may lead to increased utilization of feed nutrients and hence increased productivity (Ozioma *et al*., 2019). Studying herbal medicine can serve to validate and enhance existing local uses and can give clues to remedies with further potential. Many authors have attempted to study the economic loss due to different species of parasites in different animals all over the world including Bangladesh. Asian Development Bank (ADB) estimated that the loss of productivity of animals in terms of mortality, loss of milk and meat, generation loss and loss of production rate due to animal parasites to the extent of 50% in Bangladesh (Halder *et al*., 2019). Gastro-intestinal nematode parasitism of ruminant livestock causes significant loss in production in developing countries through mortality and reduced production of milk, meat and work potential (Gabriel *et al*., 2021)

Neem is a non-leguminous, perennial tree in the Meliaceae family and is native to Bangladesh, India, Myanmar and Pakistan growing in tropical and semitropical regions (Adjorlolo *et al*., 2016; Arnold, 2014). The major active constituent in neem is azadirachtin. It also includes nimbin, nimiodol, nimbinin, nimbidin, nimbanene, nimbadiol, nimbolide, sodium nimbinate, glucoside, nimbosterin, nimbosterol, nimbectin and fatty acid (Joshi and Prabhakar, 2021). There is a drastic reduction of parasitism in many animal species after feeding of neem leaves (Sujatha *et al*., 2022; Rahaman *et al*., 2022 and Yamson *et al*., 2019). Neem shows antibacterial, antiprotozoal, antiviral and many other properties without showing any adverse effects (Foka *et al*., 2022 and Mohideen *et al*., 2022). Therefore, the objectives of the study are to determine the efficacy of neem leaves on parasite control in lactating cows and observation of indirect effect of neem on milk production in lactating cows.

MATERIALS AND METHODS

Study area

The research was carried out in Chandi and Beradoma villages under Delduar upazila at Tangail district. The study continued for a period of 56 days with 6 Lactating cows to investigate the efficacy of the leaves of neem on gastro-intestinal nematodiasis and its impact on milk production.

Selection and grouping of the experimental animals

Feces from 10 lactating cows were collected and examined for parasite egg. Most of the animals were having parasites, but only 6 of them were selected for the experiment. The average body weight of the animal was 233.833 ± 8.540 kg. The body weight of the animal was measured by using a digital animal weighing scale. The animals were selected based on early lactation and severity of infection in terms of nematode. The similarity of live weight of the animals was also considered in the selection process.

Feeding pattern of animals

The animals were allowed to graze in the fallow land /homestead for part of the day and were also supplied with straw-based feeds (ad libitum rice straw + 500g wheat bran + ad libitum road side grasses). The feeding pattern of the animals were similar.

Marking for identification of the animals under study

Since the experimental animals were living with other animals in the smallholder farms under rural conditions, the animals were marked for easy identification and taken care of separately.

Preparation of bolus for oral administration

Leaves of neem (Azadirachta indica) were collected in the morning and washed with clean water. The leaves were then dried in the sun. The dried leaves were then ground by using a mortar and pestle. They were mixed with an equal amount of molasses to form the bolus. The amount of powder from each plant's leaves taken to make boluses was at 200 mg/kg live weight of animals. Molasses was mixed with the powder to serve as a binder and enhance palatability. Then they were made into small boluses. Boluses were made with neem and stored (one night) in colored plastic pails until use.

Layout of the experiment

The experiment is being conducted in two states:

Before treatment: Data of milk yield, body weight, and egg per gram of feces (EPG) were collected without any treatment at about 4 weeks from the selected lactating cows.

After treatment: Data of milk yield, body weight, and EPG were collected during the treatment period at about 4 weeks from the selected lactating cows.

Feeding anthelmintics to the animals

The prepared boluses were kept in different poly bags separately for each animal according to the dose of the animal (200 mg/kg live weight). The boluses were fed to cows directly after milking in the morning. Care was taken so that no spillage of the boluses occurred.

Collection of feces and examination for parasite eggs

About 10-15 g fecal samples were obtained directly from the rectum and each sample was then placed in a separate plastic bag, packed with formalin and immediately transferred to the laboratory for parasitic egg counts. Samples were collected in the morning. Firstly, the feces samples were examined for the qualitative purpose by direct smear method and if the samples were positive then the quantitative determination of helminths ova was made by the McMaster method.

Direct smear method

A drop of water was placed on the center of a clean glass slide. Small amount of feces was detached from the given sample with the help of a toothpick and spread out to form a thin smear. This can be done gently by drawing the coarse particles towards a side on the glass slide. The slide was then placed under the low power objective of a microscope for examination and counting the eggs.

McMaster method

This method is based on the principle that the eggs are floated up in a counting chamber. A special type of slide devised by McMaster is required in this technique. Two glass slides joined together and the space between them was divided to form two counting chambers of 0.15 ml capacity each in the McMaster slide. In this method, a known amount of feces (3g) was thoroughly suspended in a known volume (42 ml) of saturated salt solution (Specific gravity 1.2). The suspension was then strained through a 150 mm mesh sieve to remove the coarse particles. A portion of the suspension was withdrawn with the help of a Pasteur pipette and allowed to run into the chambers of the McMaster slide. The slide was allowed to stand for 3 to 4 minutes to allow the eggs to float. The eggs in the two chambers were counted using low power objective (10X) and eyepiece (6X) of microscope.

The number of eggs per gram of feces (EPG) was calculated by using the following formula:

$$\text{No. of eggs per gram of feces} = \frac{\text{No. of eggs in two chamber}}{0.3} \times \text{Dilution factor*}$$

$$\text{* Dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of feces}} = 45/3 = 15$$

Determination of efficacy of different treatments

Efficacy of different treatments was determined by fecal egg count reduction test using the formula mentioned below:

$$\text{Efficacy} = \frac{\text{EPG prior to treatment - EPG post treatment}}{\text{EPG prior to treatment}} \times 100$$

Records of milk

Milk yield of experimental cows was recorded for 8 weeks by farmers. The farmers were given training on record keeping and supplied with a register for keeping data. Data recording is being monitored every week at the day of fecal sample collection by researcher. Milking was done every morning at about between 07:00 am and 08:00 am.

Statistical analysis

The experimental data were analyzed using the "Statistical Package for the Social Sciences (SPSS) version 25.0" statistical program to make a comparative study between before treatment and after treatment using paired sample T-Test. Data related to milk production, body weight and parasite egg count were computed using the Stepwise Regression method.

RESULTS AND DISCUSSION

The efficacy of Neem leaves against naturally infected gastro-intestinal nematodes and its effects on the performance of lactating cows were examined. The results are summarized in the following

sections.

Effect of Neem leaves on parasite control

Data obtained from lactating cows before and after supplementation of neem leaves and after supplementation of neem leaves. From the findings, it was observed that the average EPG count was 381.250±65.234 and 300.000±45.743 before and after treatment respectively (Table 1). Statistical analysis showed that there was a significant difference ($p<0.05$) between the two states of observation. It reveals that neem leaves as herbal anthelmintics have a positive effect on the control of gastrointestinal nematodes.

Table 1 Parasitic egg count of feces of lactating cows treated with neem leaves against gastro-intestinal nematodes

Treatment	Egg per gram of feces (EPG) Mean±SD	Level of significance
Before treatment	381.250±65.234	*
After treatment	300.000±45.743	

* Significant at 5% ($p<0.05$); SD=Standard deviation

The results of the present study agree with the work of Rahaman *et al*. (2022). He decided that the use of tamarind (Tamarindus indica) and neem (A. indica) reduced fecal egg count. The work of Gathuma *et al*. (2004) reveals that herbal remedies had some efficacy against both nematodes and Monezia species of helminths. The group effect against nematodes was significantly different ($p=0.002$). Compared to an untreated control group, the efficacy against nematodes was 77, 89.8 and 90% for Myrsine africana, Albizia antihelmintica and Hilderbrantia sepalosa respectively. The study agrees with the finding of Amin *et al*. (2010) where they reported a significant ($p<0.05$) effect of neem leaf in EPG reduction at day 7, 14, 21 and 28, respectively. Amin *et al*. (2008) found that the EPG count was 62.23%, 65.77%, 56.70% and 48.05% on 3rd, 10th, 17th and 28th day, respectively in cattle using neem leaf. Reduced feed intake and reduced feed conversion efficiency occurred in nematode-infected animals. The neem leaves probably have the anti-helminthic property of due to the presence of an active alkaloid, azadirachtin, which affects with the central nervous system of parasite via inhibition of excitatory cholinergic transmission and partly blocks the calcium channel resulting in ejection parasites from host body (Qiao *et al*. 2013; Veerakumari and Priya 2006). From this observation it could be said that the herbal anthelmintics were effective in reducing nematode parasite burden and are safe to feed to the lactating cows.

Effect on anthelmintics treatment by using neem leaves on body weight

In order to observe the body weight of the dairy cattle the data were collected before and after supplementation of neem leaves. According to the results, the average body weight before and after treatment was 233.833±8.540 and 242.250±8.435 respectively (Table 2). Statistical analysis showed that there was a significant difference ($p<0.01$) between the two states of observation. It reveals that neem leaves as an herbal anthelmintic have a positive effect on the body weight gain of the animal.

Table 2 Average body weight of lactating cows treated with neem leaves against gastro-intestinal nematodes

Treatment	Average body weight (kg) Mean±SD	Level of significance
Before treatment	233.833 ± 8.540	**
After treatment	242.250 ± 8.435	

** Significant at 1% (p<0.01); SD=Standard deviation

The results of the present study agree with the work of Amin *et al*. (2008), showed increased body weight of cattle when Neem leaf was administered as anthelmintics against gastro-intestinal nematodiasis. At 28th post-treatment day, body weight increased at 3.33% and 3.70% for neem leaves and neem seeds respectively (Amin *et al*., 2008). Alam (2014) reported that, on 7th post-treatment day, egg count reduction value of neem leaves @ 25 mg, 50 mg and 100 mg per kg body weight were 5.22 %, 8 % and 10 % respectively. On the 28th post-treatment day, the efficacy of neem leaves @ 100 mg /kg body weight was 40.98 %. No substantial side effect was observed in the treated animal. Since the number of parasitic eggs reduced after providing the neem leaves, so, it can be assumed that the nutrients taken by the animal is directly utilized for body weight gain of the dairy cattle.

Effect on anthelmintics treatment by using Neem leaves on milk production

After feeding of neem leaves the data were collected from selected dairy cattle, according to the results, the average milk production before and after treatment was 2.909±0.087 and 3.093±0.084, respectively (Table 3). Statistical analysis revealed a significant difference (p<0.01) between two states of observation. It has been discovered that neem leaves, when used as a herbal anthelmintic, have a positive effect on animal milk production.

Table 3 Average milk production of lactating cows treated with neem leaves against gastro-intestinal nematodes

Treatment	Average milk production (L) Mean±SD	Level of significance
Before treatment	2.909±0.087	**
After treatment	3.093±0.084	

** Significant at 1% (p< 0.01); SD=Standard deviation

The results of the present study agree with the work of Raghavendra *et al*. (2002), who reported that fed with tree foliage from Azadirachta indica, Prosopis cineraria and Bauhinia racemosa simultaneously while grazing in range increased the milk yield by 58%. Jin *et al*. (2008) concluded that the milk production of cows supplemented with Chinese herbal medicine increased by 18.89% (p<0.01) compared to the control group during the experimental period. Milk production of the experimental group also increased by 21.42% (p<0.01) compared to the control group for 2 months after supplementation. Sarker *et al*. (2016) found that the average milk production increased by

42.11%, 52.63% and 78.95% respectively at day 7, 14, 28 after feeding of Neem leaves to the lactating cow. The use of neem leaves as a supplemented feed decreases fat deposition and subsequent reduction in saturated fatty acids, which may increase milk production. Because of the significant reduction of ruminal protozoa an increase in the total bacterial community, thereby enhancing animal performance (Kholif and Olafadehan, 2021). From this observation, it could be said that the herbal anthelmintics were effective in reducing nematode parasite burden and are safe to feed to lactating cows which is effective for body weight gain and milk production.

CONCLUSION

The supplementation of neem leaves has a positive effect on the performance of lactating cows under village conditions where cows are reared on straw based traditional diet. This may be due to the reduction of parasites and better feed utilization which may enhance the body weight as well as milk production. Medicinal plants are often cheaper and available than commercially-produced drugs. Considering the socio-economic aspect of Bangladesh, it could improve the productive performance of dairy cattle by using traditional medicinal plants. Further study is also needed to know the level of the maximum recommended dose of neem leaves in the diet of lactating cows.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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Comparative study of antihyperglycemic and antihyperlipidemic activities of aqueous extract of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves in normal and alloxan diabetic rats

MS Islam*, SK Sarkar, MM Hossain, MA Masum, T Rahman

Department of Anatomy, Histology and Physiology; Faculty of Animal Science and Veterinary Medicine; Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

*Correspondence: raselsaiful@sau.edu.bd

ABSTRACT

Effective and safer hypoglycemic agents from herbal plants are being considered as area of research as the synthetic oral anti-diabetic agents have several serious adverse effects. The aim of this research was to investigate the single and combined effect of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves on blood glucose and cholesterol in diabetic rats. In this study 60 rats were divided into 5 groups and containing 12 individuals as follows: normal control (A), diabetic control (B), Alloxan with BG treated (C), Alloxan and TK treated (D) and Group E was Alloxan, BG and TK treated. Alloxan was injected at the dose of 100mg/kg body weight intra-peritoneal to each rat to induce diabetes in groups B, C, D and E. Aqueous extract of BG and TK were fed at a dose of 300mg/kg and 500 mg/kg body weight for 21 days in group C and D, respectively and combined in group E. The body weight, blood cholesterol (mg/dl) and glucose (mg/dl) level were measured on Day 0, 7, 14 and 21. The blood sugar and cholesterol level were reduced in Group C from 178.5±11.55 mg/dl to 120.45±1.98 mg/dl and 209.11±5.24 mg/dl to 126.66±6.58 mg/dl respectively and in group D, they reduced from 150.5±1.6 to 140.17±2.49 mg/dl and 212.43±6.2 to 129.77±5.94 mg/dl respectively. But the reduction of blood sugar level and blood cholesterol level were significant in Group E from 156±4.11 mg/dl to 110.21±3.98 mg/dl and 217.17±4.34 mg/dl to 114.81±4.18 mg/dl, respectively. From these findings it concluded that the combination of BG and TK can be used as anti-hyperglycemic and anti-hypercholesterolemic agent.

Keywords: Anti-diabetic, anti-hyperglycemic, anti-hyperlipidemic

INTRODUCTION

Diabetes mellitus is a worldwide problem due to the high rates of morbidity and mortality that are connected with the condition. It may have immediate or prolonged damaging effects (Lotti & Maggi, 2023). Modern medical practices and innovative scientific discoveries have completely altered the treatment regimen. The use of glucose-lowering medications does, however, come with a number of side effects, including chronic vascular disease, renal dysfunction, liver disease, and several skin issues. These issues have prompted to consider alternate diabetic treatments with minimal or no adverse effects (Singh et al., 2022). Today, medical professionals and the public in general in both developed and developing countries alike use natural herbs and their preparations to cure disorders on the assumption that organic products are safe for body (Thikekar et al., 2021). These natural remedies encompass organic substances including flavonoids, terpenoids, glycosides, and alkaloids that exhibit a variety of anti-diabetic actions (Singh et al., 2022).

Momordica charantia (M. charantia) is a plant that has recently received the greatest interest in recent years due to its many biological benefits, which include anti-diabetic, anti-obesity, anti-inflammatory, and anti-cancer capabilities and many more (Bora et al., 2023). It also goes by

the names bitter melon or Karela in Bengali and has a wide range of pharmacological effects. Thiamine, beta-carotene, folate, riboflavin, calcium, iron, phosphorus, manganese, potassium, magnesium, zinc, and dietary fiber are just a few of the micronutrients that the bitter gourd is rich in. Regular consumption of bitter gourd juice improves physical stamina and lessens chronic fatigue. This bitter gourd's beta-carotene concentration helps to treat eye disorders and enhances vision (Mukherje & Karati, 2023). Studies have also revealed that M. charantia contains numerous phytochemicals that have hypoglycemic properties, suggesting that the plant may be useful in the treatment and control of diabetes mellitus (Oyelere et al., 2022).

Similarly, fresh leaves and branch tips from Coccinia indica (C. indica) are frequently used in Ayurvedic, Siddha, and Unani traditional medical systems throughout Asia. The common applications of this plant's many parts in folk medicine included anti-diabetic, antibacterial, anti-inflammatory, antioxidant, antimalarial, antidyslipidemic, anticancer, analgesic, antipyretic, antitussive, antinociceptive, hepatoprotective, anti-obesity, and neuroprotective functions (Padma R & Vinoth, 2022). In rats with diabetes caused by alloxan, Coccinia indica (Telakucha) leaf extract significantly reduced blood sugar and total cholesterol levels while also having regulatory effects on total erythrocyte count (TEC), total leukocyte count (TLC), and hemoglobin content (Hb) (Sarkar et al., 2020).

As both M. charantia and C. indica exert anti-diabetic properties, this study was designed to investigate the comparative efficacy of these two plants to fight against diabetes mellitus in rats' model.

MATERIAL AND METHODS

This research work was conducted in the Laboratory of Anatomy, Department of Anatomy, Histology and Physiology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka for a period of 12 months from 2021 to 2022 to evaluate the single and combined efficacy of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves on alloxan induced diabetic rats.

Collection and acclimatization of rats

Total 60 mixed male albino rats (aged 2-3 months) and weighing (70 to 100g) were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. For five experimental trials, all the rats were divided into 5 groups each containing 12 rats. Each group of rats was housed at serene bottomed wire cages arranged in rows and kept in the animal house of this department. The animals were fed with pellet at a recommended dose of 100 g/kg body weight. Drinking water was supplied ad libitum. The rats were reared in this condition for a period of two weeks to acclimatize them prior to experimental uses.

Induction of diabetes

Diabetes mellitus was induced by injecting alloxan through intraperitoneal route which increases the blood glucose level and at the same time decreased body weight. Single dose of alloxan administered intraperitoneal @100 mg/kg body weight (Junod et al., 1996). In this experiment, polyuria, polydipsia, and polyphagia after 24 hours of alloxan injection were

observed. Rats with serum glucose level ranging between 150mg/dl or above considered as hyperglycemic. At the same time the rats with cholesterol level above 200 mg/dl were considered as hypercholesterolemic (Reeves et al., 1993).

Experimental design

In this study, a total of 60 rats (12 normal rats and 48 alloxan induced diabetic rats) were used for each trial. The rats were divided into 5 groups each containing 12 individuals as follows:
Group A: Normal control
Group B: Diabetic control
Group C: Alloxan+ BG treated
Group D: Alloxan+ TK treated
Group E: Alloxan+ BG +TK treated

After 18 hours of starvation, body weights and blood glucose level were measured after acclimatization of rats. Then alloxan was injected at a dose of 100 mg/kg body weight in intraperitoneal route to each rat to induce diabetes in groups B, C, D and E. All the group of rats was reared under normal diet and water ad libitum from Day 1-10, on 10th day blood glucose level, blood cholesterol levels and body weights were measured for the first time to ensure diabetic induction as well as hypercholesterolemia. Then rats of all groups were kept for more 21 days for the treatment of hyperglycemia and hypercholesterolemia. During that period on day 0,7,14 and 21st the body weight, blood cholesterol, blood glucose level were measured. Aqueous extract of BG and TK leaves extract were fed at a dose of 300 mg/kg and 500 mg/kg body weight daily for 21 days in groups C and D respectively, and combined dose of both BG and TK in group E.

Preparation of TK Leaf Extract

TK leaves were collected from Sher-e-Bangla Agricultural University campus and dried by using freeze dry method and powdered with the help of mortar and pastel. From the powder 10% aqueous solution of TK leaf extract was prepared (Singh, et al., 2011)

Preparation of bitter melon

According to the methods of Chen and Li, (2005) unripe bitter melon fresh fruit was cut open and the seeds were removed. The extracted juice from the edible portion was frozen and completely lyophilized by continuous freeze-drying operation for 72hrs. The powder was kept in airtight containers at -700C until used.

Determination of Blood Glucose

Blood samples were collected from tail vein on 0, 7, 14 and 21st day of experiment and blood glucose was determined by using glucose oxidase-peroxidase reactive strips and a glucometer (UNI-CHECK®,Visgeneer, Taiwan). The tail was disinfected by rubbing a cotton ball soaked in Hexisol® Solution. A small amount of blood was drawn from tail vein of the rats by venipuncture with help of 13 insulin syringe and needle. At the same time the glucometer was started with a single press. Before using the test strip a new coding chip was inserted by the side of the monitor. After the monitor showed the code number the strip was inserted into the designated slot. A drop of blood was then dropped on the test zone of the strip. The result was

shown on monitor within 5 seconds of dropping the blood on the zone of the strip in mg/dl.

Determination of Total Cholesterol

Blood samples were collected from tail vein on 42nd day of experiment and blood cholesterol was determined by using a blood testing meter (EasyMate® GCU, Bioptik Technology Inc., Taiwan). The tail was disinfected by rubbing a cotton ball soaked in Hexisol® Solution. A small amount of blood was drawn from tail vein of the rats by venipuncture with help of insulin syringe and needle. At the same time the EasyMate® GCU blood testing meter was started with a single press. Before using the test strip a new coding chip was inserted by the side of the monitor. After the monitor showed the code number the strip was inserted into the designated slot. A drop of blood was then dropped on the test zone of the strip. The result was shown on monitor within 5 seconds of dropping the blood on the zone of the strip in mg/dl.

Statistical analysis

Changes in body weight, blood glucose level and blood cholesterol level of rats were compared statistically by means of one-way analysis of variance (ANOVA) test. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first comparative study of anti-hyperglycemic and anti-hyperlipidemic activities of aqueous extract of Momordica charantia and Coccinia indica leaves in normal and alloxan diabetic rat. Changes in blood glucose level of rats were summarized in the Table 1 describing that treatment of diabetic rats with BG, TK & combined treatment induced a significant decrease in fasting blood glucose levels compare with diabetic untreated group. At the day of 21, the blood sugar level was reduced in Group C from 178.5±11.55 mg/dl to 120.45±1.98 mg/dl. Likewise, in group D the blood sugar level was reduced from 150.5±1.6 mg/dl to 140.17±2.49 mg/dl. But the reduction of blood sugar level was prominent in Group E from 156±4.11 mg/dl to 110.21±3.98 mg/dl.

Table 1 Descriptive statistics of mean values of average blood sugar level (mg/dl) with standard deviation in different rat groups

Grs	Day 0	Day 7	Day 14	Day 21
	ABSL (mg/dl)	ABSL (mg/dl)	ABSL (mg/dl)	ABSL (mg/dl)
A	92.5 ±2.31	94.4 ±3.25	96.35 ± 2.86	95.10± 3.25
B	178±12.51	211.63±8.47	235.5± 6.50	255.48 ±4.14
C	178.5±11.55	160.5±5.01	129.37±6.35	120.45±1.98
D	150.5±1.65	149.5±5.01	145.33±6.35	140.17±2.49
E	156 ±4.11	139.5±3.78	127.17±1.54	110.21±3.98

Legends:
Grs: Group, Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG + TK treated; ABSL: Average Blood Sugar Level

Table 2 Descriptive statistics of mean values of blood cholesterol level (mg/dl) with standard deviation in different rat groups

Grs	Day 0 ABCL (mg/dl)	Day 7 ABCL (mg/dl)	Day 14 ABCL (mg/dl)	Day 21 ABCL (mg/ dl)
A	120.35±5.64	119.31±5.45	110.39±4.57	117.65±6.55
B	205.12±5.34	224.34±6.33	232.13±6.57	240.76±5.78
C	209.11±5.24	180.51±5.78	155.44±6.34	126.66±6.58
D	212.43±6.21	187.12±5.89	156.32±5.98	129.77±5.94
E	217.17±4.34	177.77±4.36	144.25±4.44	114.81±4.18

Legends:
Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG +TK treated; ABCL: Average Blood Cholesterol Level

Table 3 Descriptive statistics of mean values of body weight (gm) with standard deviation in different rat groups

Grs	Day 0 ABW (gm)	Day 7 ABW (gm)	Day 14 ABW (gm)	Day 21 ABW (gm)
A	302.6±5.60	300.5 ±8.42	303.33± 7.25	300.55 ±6.0
B	265.83± 8.11	236.5±8.85	221.5±7.75	215.56 ± 7.42
C	256.65±6.31	255.58±6.9	276.67±4.63	287.19±3.11
D	256.67±6.35	267.33±5.5	277.67±4.63	288.17±2.56
E	304.78 ±2.79	307.44 ±2.31	309.10 ±3.57	310.45±5.10

Legends:
Grs: Group, Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG + TK treated; ABW: Average body weight

Total cholesterol was significantly increased in alloxan induced diabetic rats, these figures were significantly decrease after BG, TK and combined treatment (Table 2).The cholesterol level was reduced in Group C from 209.11±5.24 mg/dl to 126.66±6.58 mg/dl and from 212.43±6.2 mg/dl to 129.77±5.94 mg/dl in group D after 21 days of treatment. But the reduction of blood cholesterol level was remarkable in Group E from 217.17±4.34 mg/dl to 114.81±4.18 mg/dl.

Treatment of diabetic rats with BG, TK & combined treatment induced a significant increase in body weight compare with diabetic untreated group (Table 3). After the course of 21 days of treatment with BG, TK and combination of BG & TK, the average body weight was elevated in Group C and D from 255.58±6.9 gm to 287.19±3.11 gm and 267.33±5.5 gm to 288.17±2.56 gm respectively. However, a significant increase in body weight gain was observed in group E compared with other groups from 304.78 ±2.79 gm to 310.45±5.10 gm after exposure of both BG & TK for 21 days in alloxan induced diabetic rats.

Diabetes mellitus is probably the fastest growing metabolic disease in the world. As the knowledge of multifactorial nature of this disease increases so does the need for more challenging and appropriate therapies (King et al., 1998; Dans et al., 2007). Alloxan is known for selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (Fernandes et al., 2007). Generalized increase in the level of blood glucose during diabetes have been consistently reported both in animal models and humans especially those suffering from insulin dependent diabetes mellitus (Mathew et al., 1973; Lorenzati et al., 2010).

In the present study we found that, both BG and TK extract reduced the blood glucose, and cholesterol in diabetic rats. Regarding serum glucose level, treatment of diabetic rats with bitter melon caused significant decreases in fasting and post- prandial serum glucose levels as compared to the diabetic untreated group. These results are in accordance with the previous findings (Shibib et al., 1993; Chaturvedi et al., 2004; Fernandes et al., 2007; Yuan et al., 2008; Balaraman et al., 2010; Jayasuriya et al., 2000). The present finding disagrees with the finding of Dans et al., 2007 who reported that BG had no significant hypoglycemic effect in alloxan diabetic rats. The present results elucidated a significant increase of total cholesterol concentrations in the serum of diabetic control rats as compared to normal control group. These results are in agreement with the findings that described hypo-cholesterolmic effects of BG (Newairy et al., 2002).

CONCLUSION

In conclusion, the present study reinforces our previous findings (Akhter et al., 2018a; 2018b) that BG and TK had a significant effect in reducing blood glucose as well as blood cholesterol and they may be considered as better therapeutic options for diabetes mellitus if administered together.

CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

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Effects of Papaya Leaf (Carica papaya) and Black Cumin (Nigella sativa) as an Alternative to Antibiotics on Production Index and Hematological Indicators of Broiler Chicken

MM Haque*, MAH Beg, M Begum , MM Parvin and NA Mow

Department of Poultry Science, Sher-e-Bangla Agricultural University**.** Dhaka, Bangladesh

*Correspondence: mahbeg.posc@sau.edu.bd

ABSTRACT

The purpose of the study was to determine the effectiveness of dietary supplements of papaya leaf meal (Carica papaya) and black cumin (Nigella sativa) seeds on the production index and hematological status of commercial broiler chicken. T1 (Control), T2 (antibiotic), T3 (2% PLM: Papaya Leaf Meal), T4 (1% BCS: Black Cumin Seed) and T5 (1% of each PLM & BCS) were the five treatment groups that the 150-day-old Cobb 500 straight run chicks were randomly assigned. Ten chicks were used in each of the three replications of each treatment. The findings demonstrated that feeding broilers supplements of PLM and BCS did not significantly ($p>0.05$) affect feed consumption (FC) (g), final live weight (g) and feed conversion ratio (FCR) when compared to the control and antibiotic group. FC was found to be numerically ($p>0.05$) higher in the control group than in the other groups. However, the T3 group of birds given 2% PLM had superior ultimate live weight and an improved FCR value compared to antibiotic, control, and the values of the other groups, which were statistically insignificant ($p>0.05$). The relative weight of the spleen in various groups was unaffected ($p>0.05$) by dietary supplementation with PLM and BCS. But the T3 (2% PLM) group had significantly ($p<0.05$) increased bursa weight (g). The concentrations of glucose and cholesterol were similar across all groups ($p>0.05$), while the group receiving 2% PLM supplements had relatively lower cholesterol levels (mg/dl). Moreover, the supplemented groups with PLM and BCS revealed a significant ($p<0.05$) increase in the hematological parameters red blood cell (RBC), white blood cell (WBC), lymphocyte, and packed cell volume (PCV) as compared to the antibiotic and control groups. The final live weight, FCR, immune organ weight, and blood indices were all improved in birds fed a meal supplemented with 2% PLM, leading to superior results.

Keywords: Production performance, Hematological parameters, papaya leaf meal, black cumin

INTRODUCTION

In Bangladesh, poultry production stands as one of the most significant sub-sector of the livestock industry. Despite earlier constraints, the industry managed to supply 36% of the nation's meat needs in 2019, according to the USDA. Around 1.5 to 1.6 percent of Bangladesh's GDP is currently produced by the poultry industry, according to the Bangladesh Poultry Industry Central Council (BPICC). Furthermore, the industry directly and indirectly supports almost 6 million new employment opportunities nationwide (Karmoker, 2022). Throughout the past few decades, the majority of nations have used a variety of antimicrobials in the production of chickens (Boamah et al., 2016). According to scientific evidence, the uncontrolled widespread use of antibiotics has contributed to an increase in the problem of antibiotic resistance (Furtula et al., 2010; Forgetta et al., 2012), which leads to the spread of resistant microbes and the presence of antibiotic residues in feed and the environment (Carvalho and Santos, 2016; Ronquillo and Hernandez, 2017). Because of this, using herbs and medicinal

plants as a potential substitute for antibiotic growth promoters is possible (AGPs). Herbs include a complex blend of organic compounds with immune-stimulating, antibacterial, antipyretic, and anti-inflammatory activities (Guo et al., 2003). In the current investigation, papaya leaves and black cumin seeds were utilized in place of antibiotics. Papaya is a medicinal plant contains a number of phytochemicals, including lycopene and polyphenols (Kale et al., 2003).The proteolytic enzymes papain and chymopapain, which have the ability to degrade protein, are abundant in papaya leaves and helpful in preventing digestive issues and intestinal worms (Burkill, 1985). Moreover, papaya leaves contain carotene, a provitamin A that can provide around 18 – 50 IU and is a promising natural source of xanthophyl. Iron, calcium, phosphorus, vitamin C, and vitamin E are all found in papaya leaves. In addition, the leaves have a crude protein content of 20.88%, 0.99% calcium, 0.47% phosphorus, and 2912 kcal/kg gross energy. Malaria and dengue illness are treated using papaya leaves. Papaya leaves have been shown to greatly reduce the risk of coccidiosis (AL-Fifi, 2007; Nghonjuyi, 2015). It has been discovered that papaya leaf contributed to the blood's color, which in the supplemented study had the maximum quantity of RBC, hemoglobin, platelets, and PCV (Agboola et al., 2018). Several researches demonstrate that broilers treated with papaya leaf could obtain higher growth performance and immunological response (Sorwar et al., 2016; Oloruntola et al., 2018). Black cumin often referred to as "Kalojeera" locally, and is being recognized as a miracle plant. Black cumin holds the top spot among herbal medications as a result of its remarkable therapeutic abilities. Black cumin has a wide range of medicinal potentials, including antibacterial, antihypertensive, anticancer (Padhye et al., 2008), immune-modulatory, analgesic, antimicrobial, antihelminthic, anti-inflammatory, gastro protective, and renal protective effects (Zaoui et al., 2000). According to several research, black cumin seed was effective at promoting weight gain, FCR (Mansoori et al., 2006; Khan et al., 2012), feed intake, dressing percentage, and the weight of various internal organs (Durrani et al., 2007). On the basis of this background, the trial was designed to investigate the effects of papaya leaf meal and black cumin seed as potential replacements for antibiotic growth promoters on the growth performance, immunological condition, and blood indices of broiler chickens.

MATERIALS AND METHODS

Statement of the Experiment

To determine the viability of using papaya leaf and black cumin in commercial broiler diet on growth performance, immune status, and blood indices of broiler chickens, research was conducted in the experimental trial shed at Sher-e-Bangla Agricultural University Poultry Farm, Dhaka, using 150 day-old Cobb 500 straight run commercial broiler chicks from a single hatch over a 28-day period from February 19 to March 19, 2019.

Experimental Materials and Design

From a reputable hatchery, 150-day-old Cobb 500 straight run commercial broiler chicks were collected. Following standard brooding practices, they were kept in an electric brooder for seven days. Only the most basic nutrients in farm made starter (3027 ME Kcal/Kg & 21.86% CP) and grower ration (3152 ME Kcal/Kg & 21.12% CP) was offered. One hundred and fifty (150) DOCs were divided into five treatment groups at random after four days, with three replicates in each group (10 chicks). Good-quality papaya leaves and black cumin were gathered from papaya

gardens and nearby markets, respectively. Following collection, papaya leaves were properly cleaned, allowed to air dry beneath a shed for seven days, and then crushed in a wooden mortar and pestle. Then, using a sieve, the leaf veins were separated. Moreover, fresh drinking water was used to wash and adequately dry the black cumin. Both components were suspended in air. Until utilized, both ingredients were kept separate in an airtight container.

The following experimental diets were created
T1 = Basal diets (control)
T2 = Basal diets + Antibiotics ((Doxivet ® - 1g/2 litre of drinking water)
T3 = 2% PLM (2 kg of PLM/100 kg of the feeds)
T4 = 1% BCS (1 kg of BCS/100 kg of the feeds)
T5 = 1% PLM & 1% BCS (1 kg PLM & 1 kg BCS/100 kg of the feeds)

Management Procedures

The experimental shed was cleaned, sanitized, and disinfected. The open-sided, south-facing shelter allows cross ventilation. Over the duration of the experiment, averages for room temperature (290C) and relative humidity (73%) were noted. Rice husk was used as the litter material, and a depth of 6 cm of litter was formed. Litter was stirred at the end of each day to minimize the buildup of noxious substances and to lessen parasite infestation. The birds were given rations and free access to clean water. Water was provided twice day, while food was provided three times daily. The shed was equipped with lighting at night. For the first two days, there was 24 hour light. Ranikhet Disease, Infectious Bronchitis and Infectious Bursal Disease (IBD) vaccines were given to chicks in time.

Recorded Parameters

Information was gathered on the weekly live weight, weekly feed consumption, and chick deaths to calculate survivability rate. The FCR was determined using the ultimate live weight and total feed consumed by each bird in each replication. At the end of the experiment, In order to assess the weight of the broiler chicken's spleen and bursa, three birds from each replicate that had been fasting for 12 hours were randomly chosen and slaughtered. The wing vein was used to collect blood samples (about 3 mL each) that were used to evaluate the hemato-biochemical characteristics of the birds before slaughter. Ethylene diethyltetraacetic acid (EDTA) was added to the blood sample tubes; however a portion of each sample was collected without EDTA to obtain serum. Within an hour of being collected, samples were brought to the lab for analysis. Analysis from each replication to measure glucose and cholesterol level, hemoglobin, RBC, WBC, PCV level and lymphocyte percentage.

Analytical Statistics

The data was statistically analyzed using the statistical package for social sciences (SPSS) version 16 by applying one way ANOVA. Duncan's multiple comparison test was used to evaluate differences between means, with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Production Index

Final Live Weight

The effects of treatments on final live weight (g/bird) were not significant ($p>0.05$), according to the data reported in Table 1. However, the 2% PLM treated group was shown to have an insignificantly ($p>0.05$) higher Final live weight (1559.57g) of birds than the other groups. These findings agreed with those reported by Unigwe et al. (2014), who discovered that dietary PLM supplementation had no noticeable ($p>0.05$) effect on the average final body weight of broiler chicken. Moreover, these results were at conflict with Ebenebe et al. (2011) who found that adding PLM to the diet of broiler chickens resulted in greater weight gain than in the control group. According to Jahan et al. (2015), adding BCS Meal to the diet increased the live weight of broiler chickens by a significant ($p<0.05$) amount when compared to the control group.

Total Feed Consumption

No significant ($p>0.05$) differences in the feed intake of broiler chickens different treatment groups were revealed by the data in Table 1. Insignificantly ($p>0.05$), the control group consumed more feed per bird (2212.60g), while the 1% BCS (T4) treatment group received less feed per bird (2175.23g). These findings concur with those of Bolu et al. (2009) who noted that adding papaya leaf to broiler diets had no significant impact ($p>0.05$) on feed intake and Guler et al. (2006) who also noted that adding black cumin seed to broiler diets had no significant impact ($p>0.05$) on feed consumption of broiler chicken. However, other researchers (Rumokoy et al., 2016) came to the opposite conclusion, finding that dietary papaya extract supplementation had a greater significant ($p<0.01$) interaction on feed intake.

Feed Conversion Ratio

According to the data in Table 1, the treatments' effects on the feed conversion ratio (FCR) in broiler chickens were not statistically significant ($p>0.05$). Numerically ($p>0.05$) the control group FCR was higher (1.46), while the birds supplemented with 2% PLM had a lower FCR (1.41). These outcomes were in line with those of Unigwe et al. (2014), who came to the conclusion that FCR in broilers supplemented with dried PLM at 5%, 10%, and 15% inclusion levels were not significantly ($p>0.05$) different between all treatment groups and compared to that of the control group. Moreover, results from other studies (Majeed et al., 2010; Saeid et al., 2013) revealed that the dietary supplementation of Black cumin seed did not significantly affect FCR in broiler chicken.

Survivability

According to the data in Table 1, adding PLM and BCS to the diet had no negative effects on the broiler chicken's survival rate (100%). During the study period, No mortality was found.

Immune Organs

Table 2 showed that adding papaya leaf meal and black cumin seed to broiler ration did not significantly ($p>0.05$) affect spleen weight (g), but did significantly ($p<0.05$) affect bursa weight. Insignificantly ($p>0.05$) the lowest value was in the control group (1.78g), whereas the greatest spleen weight (2.28g) was recorded in the T4 group. In this case, significantly ($p<0.05$) the highest bursa weight (2.50g) was found in the 2% PLM supplemented group and lowest (1.67g)

in the control group. In relation to the findings, a number of researchers reported that the addition of papaya leaf (Battaa et al., 2015; Haruna and Odunsi, 2018) and black cumin (Toghyani et al., 2010) seed supplements increased spleen and bursa weight in comparison to the control group.

Serum Biochemical Parameters

Moreover, Table 3 showed that there were no significant differences ($p>0.05$) in the concentrations of glucose and cholesterol (mg/dl) throughout the various dietary groups Mean value of glucose and cholesterol was 196.89mg/dl and 172.22mg/dl respectively. The 2% PLM supplementation group (T3) had lower cholesterol levels (169.11 mg/dl) than the antibiotic and control groups, but there was no statistically significant ($p>0.05$) difference among the groups. The results of the current study contradicted those of other researchers (Akhtar et al., 2003; Khadr and Abdel-Fattah, 2006), who claimed that dietary papaya leaf and black cumin supplementation effectively decreased serum glucose level. These findings are in line with those of other studies (Zetina-Esquivel et al., 2015), which found that supplementing with PLM reduced serum cholesterol levels in hypercholesterolemic rats.

Table 1 Production index of broiler chicken supplemented with Papaya leaf meal, Black cumin seed and antibiotic.

Treatments	Final Live Weight (g)/bird	Total Feed Consumption (g)/bird	FCR	Survivability (%)
T_1	1511.90 ± 10.11	2212.60 ± 11.32	1.46±.01	100.00 ± 00
T_2	1541.33 ± 22.25	2193.70 ± 17.50	1.42±.02	100.00 ± 00
T_3	1559.57 ± 26.404	2198.43 ± 10.45	1.41±.03	100.00 ± 00
T_4	1532.77 ± 27.16	2175.23 ± 7.04	1.42±.02	100.00 ± 00
T_5	1520.27 ± 10.58	2184.03 ± 10.52	1.44±.02	100.00 ± 00
Mean ± SE	1533.17^{NS} ± 9.00	2192.80^{NS} ± 5.63	1.43^{NS}±.01	100.00 ± 0.00

Here, T_1= (Control); T_2= (Antibiotic), T_3= (2% PLM supplementation); T_4= (1% BCS supplementation) and T_5= (1% PLM & 1% BCS supplementation); NS= Non-significant ($p>0.05$); SE= Standard Error

Table 2 Effects of Papaya leaf meal, Black cumin and antibiotic supplementation to broiler diet on some immune organs.

Treatments	Spleen weight (g)	Bursa weight (g)
T_1	1.78 ± 0.12	1.67 ± 0.17^{b}
T_2	1.83 ± 0.14	2.22 ± 0.25^{ab}
T_3	1.940± 0.29	2.50 ± 0.41^{a}
T_4	2.28 ± 0.17	1.72 ± 0.22^{ab}
T_5	1.94 ± 0.16	2.22 ± 0.12^{ab}
Mean ±SE	1.96^{NS} ± 0.08	2.07 ± 0.12*

Here, T_1= (Control); T2= (Antibiotic); T3= (2% PLM supplementation); T_4= (1% BCS supplementation) and T_5= (1% PLM & 1% BCS supplementation); * = Significant ($p<0.05$); NS= Non-significant ($p>0.05$) $^{a, b}$ Mean values with different superscripts within the same column differ significantly; SE= Standard Error

Table 3 Effects of Papaya leaf meal, Black cumin seed and antibiotic supplementation on serum biochemical concentration of broiler chickens.

Treatments	Glucose (mg/dl)	Cholesterol (mg/dl)
T_1	199.11 ± 3.65	176.00 ± 3.85
T_2	202.11 ± 5.18	172.11 ± 4.72
T_3	196.11 ± 2.12	169.11 ± 5.31
T_4	192.11 ± 1.47	170.67 ± 5.21
T_5	195.00 ± 2.06	173.22 ± 5.39
Mean ± SE	196.89^{NS} ± 1.46	172.22^{NS} ± 2.13

Here, T_1= (Control); T2= (Antibiotic); T3= (2% PLM supplementation); T_4= (1% BCS supplementation) and T_5= (1% PLM & 1% BCS supplementation); NS= Non-significant (p>0.05); SE= Standard Error

Table 4 Effects of Papaya leaf meal, Black cumin seed and antibiotic on blood parameters of broiler chickens.

Treatment group	Hb (g/dl)	RBC (million/cumm)	PCV %	WBC ($\times10^3$/cumm)	Lymphocytes%
T_1	10.63 ± 0.30	3.61 ± 0.10^{c}	36.99 ± 0.32^{c}	7.48 ± 0.44^{c}	23.56 ± 0.63^{b}
T_2	10.97 ± 0.36	3.98 ± 0.08^{b}	35.60 ± 0.45^{c}	7.77 ± 0.50^{bc}	24.00 ± 0.82^{b}
T_3	11.93 ± 0.57	4.51 ± 0.16^{a}	42.00 ± 1.18^{a}	9.53 ± 0.08^{a}	35.22 ± 2.60^{a}
T_4	11.53 ± 0.44	4.39 ± 0.10^{a}	38.41 ± 1.11^{bc}	8.03 ± 0.34^{bc}	31.11 ± 2.14^{a}
T_5	11.32 ± 0.40	4.28 ± 0.09^{ab}	41.00 ± 1.94^{ab}	8.86 ± 0.41^{ab}	32.67 ± 1.85^{a}
Mean± SE	11.28^{NS} ± 0.19	4.15 ± 0.07*	38.80 ± 0.61*	$8.33 \pm 0.20^{*}$	29.31 ± 1.04*

Here, T_1= (Control); T2= (Antibiotic); T3= (2% PLM supplementation); T_4= (1% BCS supplementation) and T_5= (1% PLM & 1% BCS supplementation); * = Significant (p<0.05); NS= Non-significant (p>0.05); $^{a, b, c}$ Mean values with different superscript letters within the same column differ significantly; SE= Standard Error

Hematological Parameters

Table 4 showed that, with the exception of Hb (g/dl), papaya leaf meal and black cumin seed in the feed had a significant (p<0.05) effect on the RBC (million/cumm), PCV (%), WBC (×103/cumm) and lymphocyte (%) concentrations of broiler chicks in the treatment group. RBC, WBC, lymphocyte and PCV values were greater (p<0.05) in the T3 (2% PLM), T4 (1% BCS), and T5 (1% of both PLM and BCS) groups than in the antibiotic (T2) and control (T1) groups. RBC (4.51 million/cumm), PCV (42.00%), WBC (9.53×103/cumm) and lymphocyte (35.22%) values were highest in the T3 group. Agboola et al. (2018) found that broiler chicken diets incorporating papaya leaf meal showed the greatest values of packed cell volume, red blood cells, and hemoglobin in all the papaya leaf included treatments, were in agreement with these findings. On the other hand, these results were at conflicts with those of earlier studies, which found that dietary supplements of Carica papaya leaf and black cumin did not significantly differ (p>0.05) from control groups in terms of the general blood parameters (Jamroz and Kamel, 2002; Bolu et al., 2009).

CONCLUSION

According to the results of this study indicated that birds fed 2% PLM had numerically higher body weight, improved FCR, and lower serum cholesterol levels. Moreover, hematological indicators improved and the weight of the immune organ (bursa) increased significantly as compared to the control, antibiotic, and other supplemented groups receiving Black cumin seed and Papaya leaf meal diet.

CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

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Effect of feeding cinnamon and garlic as an alternative to antibiotic on growth performance and carcass characteristics in broiler

M Akter[1]*, M Asaduzzaman[2] and FY Sumi[1]

[1]Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka
[2]Department of Dairy Science, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

*Correspondence: makter.angb@sau.edu.bd

ABSTRACT

The experiment was conducted to evaluate the potential of feeding cinnamon and garlic on growth performance, carcass yield and immune parameters in commercial broiler. A total of 225 one-day-old Lohman meat (Indian river) broiler chicks have an average body weight of 40±0.50 g were divided into 5 dietary treatment groups. The treatments were T_0= control (basal feed), T_1= basal feed + antibiotic, T_2= basal feed + 0.2% cinnamon, T_3= basal feed + 0.2% garlic and T_4= basal feed + 0.2% cinnamon + 0.2% garlic. The average live weight and body weight gain were significantly ($P<0.05$) increased in the 0.2% cinnamon + 0.2% garlic treated group compared with others. Improved FCR was observed in birds fed a combined addition of 0.2% cinnamon and 0.2% garlic with the basal diet than the other treatment groups. All the supplemented group significantly increased the proportion of breast meat compared to control, while wing and back meat was higher in the T_2, T_3 and T_4 supplemented group compared to T_0 and T_1 group ($P<0.05$). Dietary supplementation of T_2, T_3 and T_4 also increased the weight of liver, heart, neck, gizzard and giblet ($P<0.05$). Dietary supplementation of T_1, T_2, T_3 and T_4 significantly reduced the concentration of blood WBC and Granulocyte. Taken together, it can be concluded that the use of 0.2% cinnamon and 0.2% garlic in broiler diet separately or combined resulted in improved growth performance, carcass yields, and immunity in broiler chickens and therefore can be recommended as alternatives to antibiotics.

Keywords: Cinnamon, garlic, growth performance, broiler, antibiotic alternatives

INTRODUCTION

Sub-clinical application of in-feed antibiotics in animal diets has appeared as a controversial issue worldwide and is facing reduced social acceptance due to the emergence of residues and resistant strains of bacteria. Scientific evidence recommends that the massive use of these compounds has led to increased the problem of antibiotic resistance (Forgetta et al., 2012; Furtula et al., 2010), and the presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez Ronquillo and Angeles Hernandez, 2017), compromises human and animal health (Diarra et al., 2010). As a results new alternatives are being investigated for livestock producers, among which phytogenic and herbal products have been given considerable awareness as possible in-feed antibiotics substitutions. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions.

Cinnamon (*Cinnamomum zeylanicum*) commonly known as “dalchini” is one of the earliest medicinal plants, widely used as condiments in India and indigenous to Sri Lanka and South

India (Jakhetia et al., 2010). The principal chemical components of cinnamon are cinnamaldehyde, trans-cinnamaldehyde (Cin) and eugenol, which are present in the essential oil and contribute to the fragrance and various biological activities (Chang et al., 2013). Studies have revealed that cinnamon possesses appetite and digestion-stimulant properties, anti-bacterial properties (Chang et al., 2001), antioxidant properties and other medicinal properties like anti-ulcer, anti-diabetic, anti-inflammatory (Jakhetia et al., 2010). Cinnamon (*Cinnamomum cassia*) is known as an appetite and digestion stimulant and its antimicrobial properties are mainly related to its cinnamaldehyde content followed by eugenol and carvacrol (Tabak et al., 1999). Cinnamaldehyde and eugenol have been indicated to possess antibacterial activity against a wide range of bacteria (Chang et al., 2001), antioxidant properties (Gurdip et al., 2007) and inhibitory properties against *Aspergillus flavus* (Montes-Belmont and Carvajal, 1998). Recent studies have also observed that cinnamon powder, cinnamaldehyde alone or in combination with other essential oils had a wide line-up of beneficial effects in poultry. Some of those effects include increased feed intake, improved performance, feed efficiency and health status, increased breast meat yield (Al-Kassie, 2009), increased pancreatic and intestinal lipase activity (Kim et al., 2010), improved growth performance and meat quality (Sang-Oh et al., 2013), protection against pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Salmonella sp.*, *Helicobacter pylori* and *Parahemolyticus* (Chang et al., 2001).

Garlic (*Allium sativum*) is an issue of considerable interest as a medicine and therapeutic agent worldwide since ancient times. The main pharmacological effects of garlic are ascribed to its organosulphur compounds (Tapiero et al., 2004). Freshly crushed garlic contains allicin, alliin, ajoene, diallylsulfide, dithiin, S- allylcysteine. Garlic as an accepted feed additive in poultry nutrition may be of great advantage and importance particularly for broiler growers. This is outstanding to their antibacterial, anti-inflammatory, antiseptic, anti-parasitic and immunomodulatory properties of garlic. In vitro studies have discovered that garlic has antibacterial, antifungal, antiparasitic, antiviral (Ankri and Mirelman, 1999), antioxidant (Prasad et al., 1995), as well as antithrombotic, vasodilatory and anticancer (Agarwal, 1996) activities. It has benefits in lowering total plasma cholesterol, reducing blood pressure and decreasing platelet aggregation (Sterling and Eagling, 2001). Additionally, garlic is exact rich in aromatic oils, which upsurge digestion and positively influenced respiratory system being breathe in into air sacs and lungs of birds. Also, it was found that garlic has resilient antioxidative properties (Gardzielewska et al., 2003).

A number of studies have been conducted to examine the consequence of garlic (Demir et al., 2003; Kim et al., 2009) and cinnamon powder (Chen et al., 2008a, 2008b; Park, 2008) on broiler performance and meat quality but the results have not been compatible. Moreover, research on the combined use of cinnamon and garlic in performance of broiler production is still limited in Bangladesh. We hypothesized that cinnamon and garlic can assure as alternatives for antibiotics as pressure to eliminate growth promotant antibiotic use increases in Bangladesh. Thus, this study designed at assessing the potential of feeding of cinnamon

and garlic as antibiotic alternative on growth performance and immune responses in broilers.

MATERIALS AND METHODS

The research work was conducted at Sher-e-Bangla Agricultural University, Poultry Farm, Dhaka, with 225 one-day-old chicks for a period of 28 days from 27th July to 23th August, 2022 to investigate the separate and combined effect of cinnamon and garlic on growth performance, carcass traits and immune parameters of commercial broiler.

Preparation of experimental house

The broiler shed was an open-sided house with a concrete floor. The experimental room was properly cleaned and washed by using tap water. All the equipment of the broiler house was cleaned and disinfected. The house was disinfected by n-alkyl dimethyl benzyl ammonium chloride (TimsenTM) solution before starting the experiment. After proper drying, the house was divided into pens as per the layout of the experiment by polythene sheet so that air cannot pass from one pen to another. Before placement of chicks, the house was fumigated by formalin and potassium permanganate @ 500 ml formalin and 250 g potassium permanganate (i.e. 2:1) for 35 m^3 experimental area. Fresh, clean and sun-dried rice husk was used as shallow litter to absorb moisture from fecal discharge of broiler chicken. About 250 g calcium oxide powder was mixed with rice husk in every pen as disinfectant. Electric brooder was used to brood chicks. Brooding temperature was adjusted (below 35° C) with house temperature. Electric fans were used as per necessity to save the birds from the heat stress. The brooding temperature was checked every 2 hours later by digital thermometer to maintain the temperature of the brooder. Due to wire-net cross ventilation was easy to remove polluted gases from the farm. Daily room temperature (℃) and humidity were recorded with a digital thermo-hygrometer.

Experimental layout, birds and dietary treatments

A total of 225 one-day-old broiler chicks were divided into 5 experimental groups with 3 replications of 15 chicks each using a completely randomized block design. Experimental layout is presented in Table 1. The "Lohman Meat (Indian River)" strain having 40.00±0.50 g average body weight was purchased from Kazi farm limited hatchery, Gazipur, Dhaka. Starter and grower commercial Kazi broiler feed were procured from the local market. Feed was supplied 4 times daily by following Indian River Management Manual and *ad libitum* drinking water 2 times daily. The composition of the starter and grower diet which was collected from Kazi farm limited company is given in Table 2.

Experimental materials

The collected chicks were transferred to the university poultry farm and held in electric brooders similarly for 7 days by retaining standard brooding protocol. During brooding time only basal diet was given, no cinnamon/garlic was used as treatment. The chicks were supplied glucose water with vitamin-C to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers

to start feeding for the first 24 hours. After seven days, chicks from brooders were distributed randomly in dietary treatments. The birds were vaccinated on the proper schedule against new castle disease, infectious bronchitis and infectious bursal disease (Table 3). Vitamin-B complex, vitamin-A, D_3, and E were used against deficiency diseases. Electromin and Vitamin-C also used to save the birds from heat stress. After 28 days of nursing and feeding, data were collected for the following parameters: feed intake, live weight, body weight gain, feed conversion ratio, carcass characteristics, and total blood count.

Collection of cinnamon and garlic powder

The cinnamon and garlic powder of Pran Company were purchased from Super shop. The cinnamon and garlic powder were supplied to the birds after mixing with feed according to the desired treatments.

Recorded parameters

Weekly live weight, weekly feed intake and death of chicks to calculate mortality percent were taken during the experimental period. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter carcass weight and gizzard, liver, spleen, bursa, intestine and heart were measured from each broiler chicken. The dressing yield was calculated for each replication to find out the dressing percentage.

At the end of the experiment, blood sample was collected randomly from each replication of every treatment. About 2mL blood was collected from wing vein with syringe in a vacutainer. Vacutainer contains EDTA solution which prevents blood coagulants. Few hours after collection the blood sample was tested by Auto Blood Analyzer in the laboratory.

Statistical analysis

Total data were compiled, tabulated and analyzed according to the objectives of the study. Excel Program was practiced for preliminary data calculation. The collected data was subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS version 16.0). Differences between means were tested using Duncan's multiple comparison test, LSD and significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The research work was accompanied to investigate the effect of feeding cinnamon and garlic on growth performance of commercial broiler. The production performances of broiler chicken were evaluated by average live weight, average feed consumption (FC), weekly feed consumption, feed conversion ratio (FCR), average body weight gain, weekly body weight gain and survivability of birds. Carcass characteristics were taken by dressing percentage (DP), carcass weight and relative weight of giblet organs. The results of this research work are given and discussed below:

Table 1. Layout of the experiment

Treatments	Arrangement of treatments	Replications			Total number of birds
		1	2	3	
T_0	Basal feed (ready-made feed)	15	15	15	45
T_1	Basal feed + Antibiotic	15	15	15	45
T_2	Basal feed + 0.2% Cinnamon	15	15	15	45
T_3	Basal feed + 0.2% Garlic	15	15	15	45
T_4	Basal Feed + 0.2% Cinnamon + 0.2% Garlic	15	15	15	45
Total					**225**

Table 2. Chemical composition of the basal diet (starter and grower)

Parameter	Starter diet (0-7 days)	Grower diet (8-28 days)
Protein (%)	21.0	19.0
Fat (%)	6.0	6.0
Fiber (%)	5.0	5.0
Ash (%)	8.0	8.0
Lysine (%)	1.20	1.10
Methionine (%)	0.49	0.47
Cysteine (%)	0.40	0.39
Tryptophan (%)	0.19	0.18
Threonine (%)	0.79	0.75
Arginine (%)	1.26	1.18

Table 3. The vaccination schedule

Age of birds	Vaccine	Name of vaccine	Route of administration
2 days	Infectious Bronchitis+ Newcastle Disease	CEVAC BIL Vaccine	One drop in one eye
9 days	Gumboro	CEVAC IBD L Vaccine	One drop in one eye
16 days	Gumboro	CEVAC IBD L Vaccine	Drinking water
19 days	Newcastle Disease	CEVAC NEW L Vaccine	Drinking water

Table 4. Effects of Cinnamon and Garlic on growth performances of broiler chicken

Treatments	Average Live Weight (g/bird)	Average BWG (g/bird)	Average FI (g/bird)	Final FCR	Dressing (%)	Survivability (%)
T_0	1942.67^{bd}±0.32	1902.67^{c}±0.33	2549.58±1.00	1.34±0.012	72.57±0.20	100.00±0.00
T_1	1947.33^{bd}±0.40	1907.33^{c}±0.41	2550.00±0.30	1.34±0.020	74.87±0.10	95.56±0.50
T_2	1987.33^{bc}±0.07	1947.33^{b}±0.07	2589.95±0.50	1.33±0.030	75.95±0.06	100.00±0.00
T_3	1979.00^{b}±0.72	1939.00^{bc}±0.73	2578.87±0.80	1.33±0.031	75.99±0.32	100.00±0.00
T_4	2085.00^{a}±1.59	2045.00^{a}±1.60	2678.95±0.50	1.31±0.020	76.21±0.24	100.00±0.00
Level of Significance	*	*	NS	NS	NS	NS

Here, T_0 = Control (basal feed), T_1 = Basal feed + Antibiotic, T_2 = Basal feed + 0.2% Cinnamon, T_3 = Basal feed + 0.2% Garlic, and T_4 = Basal feed + 0.2% Cinnamon + 0.2% Garlic. Values are mean ± SE (n=45) one way ANOVA (SPSS, Duncan method). * Significant difference ($P<0.05$). NS: Non-significant. SE= Standard Error. ^{abcd}: Values bearing different letters within each column differ significantly.

Table 5. Effects of Cinnamon and Garlic on carcass characteristics of broiler chickens

Treatments	Breast (g)	Thigh (g)	Wing (g)	Drumstick (g)	Back (g)
T_0	400.50^{d}±1.00	96.00±2.33	90.86^{b}±2.00	75.55±0.40	210.00^{c}±2.00
T_1	476.77^{c}±2.50	95.50±1.09	95.25^{b}±0.40	75.60±1.64	208.70^{c}±1.80
T_2	490.50^{b}±1.50	100.86±1.00	127.75^{a}±0.90	77.80±0.75	230.55^{b}±0.50
T_3	500.30^{b}±1.17	102.50±0.80	129.50^{a}±1.70	80.70±0.78	245.00^{b}±1.00
T_4	550.80^{a}±1.40	110.90±0.83	140.30^{a}±1.70	87.00±0.50	290.80^{a}±1.77
Level of significance	*	NS	*	NS	*

Here, T_0 = Control (basal feed), T_1 = Basal feed + Antibiotic, T_2 = Basal feed + 0.2% Cinnamon, T_3 = Basal feed + 0.2% Garlic, and T_4 = Basal feed + 0.2% Cinnamon + 0.2% Garlic. Values are mean ± SE (n=45) one way ANOVA (SPSS, Duncan method). * Significant difference (P<0.05). NS: Non-significant. SE= Standard Error. ^{abcd}: Values bearing different letters within each column differ significantly.

Table 6. Effects of Cinnamon and Garlic on internal organs of broiler chickens under different treatment groups

Treat-ment	Liver (g/bird)	Heart (g/bird)	Neck (g/bird)	Gizzard (g/bird)	Giblet (g/bird)	Intestine (g/bird)	Spleen (g/bird)	Bursa (g/bird)
T_0	45.70^{c}±0.70	9.50^{b}±0.55	40.80^{c}±3.30	25.14^{b}±0.14	121.14^{c}± 1.00	128.30±0.40	2.00±0.20	2.00±0.70
T_1	45.55^{c}±1.00	9.75^{b}±0.30	41.00^{c}±1.07	28.85^{b}±1.20	125.15^{c}±0.78	130.00±1.00	2.20±0.20	2.10±0.30
T_2	49.50^{bc}±0.50	11.00^{a}±0.70	44.50^{b}±5.51	40.15^{a}±0.40	145.15^{b}±0.40	120.50±1.30	2.05±0.40	2.10±0.46
T_3	51.55^{ab}±1.20	11.70^{a}±0.40	45.38^{b}±3.70	42.10^{a}±0.70	150.73^{ab}±0.87	122.00±0.55	2.22±0.30	2.15±0.70
T_4	59.80^{a}±0.60	12.80^{a}±0.25	48.90^{a}±1.77	45.20^{a}±2.00	166.70^{a}±1.30	126.55±1.50	2.28±0.40	2.10±0.44
Level of significance	*	*	*	*	*	NS	NS	NS

Here, T_0 = Control (basal feed), T_1 = Basal feed + Antibiotic, T_2 = Basal feed + 0.2% Cinnamon, T_3 = Basal feed + 0.2% Garlic, and T_4 = Basal feed + 0.2% Cinnamon + 0.2% Garlic. Values are mean ± SE (n=45) one way ANOVA (SPSS, Duncan method). * Significant difference (P<0.05). NS: Non-significant. SE= Standard Error. ^{abc}: Values bearing different letters within each column differ significantly.

Table 7. Effects of Cinnamon and Garlic on immune parameters of broiler chicken under different treatment groups

Treatment	WBC ($x10^9$/L)	Lymphocyte($x10^9$/L)	Granulocyte($x10^9$/L)
T_0	16.50^{a}±0.11	2.00^{abc}±0.19	11.20^{a}±0.10
T_1	9.00^{b}±0.45	2.50^{a}±0.25	4.11^{b}±0.20
T_2	9.00^{c}±0.20	3.00^{a}±0.20	7.00^{c}±0.10
T_3	9.50^{c}±0.22	2.66^{b}±0.10	7.70^{d}±0.14
T_4	5.00^{d}±0.10	2.05^{c}±0.13	3.50^{e}±0.10
Level of significance	*	*	*

Here, T_0 = Control (basal feed), T_1 = Basal feed + Antibiotic, T_2 = Basal feed + 0.2% Cinnamon, T_3 = Basal feed + 0.2% Garlic, and T_4 = Basal feed + 0.2% Cinnamon + 0.2% Garlic. Values are mean ± SE (n=45) one way ANOVA (SPSS, Duncan method). * Significant difference (P<0.05). SE= Standard Error. abcde: Values bearing different letters within each column differ significantly.

Growth performance

Average live weight

The data presented in Table 4 showed the effect of cinnamon and garlic on growth performances of broiler. The relative average live weight (g) of broiler chickens at the end of 4^{th} week in the dietary group T_0, T_1, T_2, T_3 and T_4 were 1942.67±0.32, 1947.33±0.40, 1987.33±0.07, 1979.00±0.72 and 2085.00±1.59 respectively (Table 4). There was a significant ($P<0.05$) difference between the T_4 and control as well as the others. Moreover, there was no significant difference between the antibiotics group and the control, cinnamon and garlic-treated group. The maximum live weight was found in T_4 (2085.00±1.59) and the lowest result was in T_0 (1942.67±0.32) group. Park (2008) also showed a remarkably higher body weight for broilers fed diets containing 3.0% cinnamon powder compared to those fed the control feeds, which is in alike with the findings of Chen et al. (2008a) who described that broiler supplied cinnamon extract added diets had significantly higher average daily gain and lower feed to gain ratio in the whole 6-week period compared with the control. Al-Kassie (2009) found positive effects of ground thyme and cinnamon on the live weight gain and enhancement of the health of broiler chickens, with the other performance traits, feed conversion ratio, and feed intake. Toghyani et al. (2011) showed that dietary inclusion of cinnamon @ 2 g/kg diet improved body weight significantly and revealed that it could be an alternative to antibiotics in broilers. Therefore, the addition of cinnamon and garlic in broiler diet increases the average live weight and a significantly increased weight is observed when cinnamon and garlic are added combined.

Average body weight gain

The data presented in Table 4 also showed that the effect of feeding cinnamon and garlic on total body weight gain (gram per broiler chicken) broiler. From the table, it is clear that there is significant difference in total body weight gain among the treatments. The relative total body weight gain (g) of broiler chickens in the dietary group T_0, T_1, T_2, T_3 and T_4 were 1902.67±0.33, 1907.33±0.41, 1947.33±0.07, 1939.00±0.73 and 2045.00±1.60 respectively. The highest result was found in T_4 (2045.00±1.60) and the lowest result was in T_0 (1902.67±0.33) group. Moreover, the individual cinnamon (T_2) and garlic (T_3) group also shows significantly ($P<0.05$) better results than the control and antibiotic groups. Similarly, Gbenga et al. (2009) showed that weight gain, average feed intake and FCR of the birds receiving dietary garlic were not significantly increased, but broiler chicks fed garlic-supplemented diets had insignificantly higher weight gain than those fed the control diet which was higher at a high level of garlic supplementation. This study results highlighted that the combination of cinnamon and garlic significantly increases the average body weight gain in broiler.

Feed intake

Data presented in Table 4 showed that the result of different treatments on final feed consumption (gram per broiler chicken) were not significant ($P>0.05$). The mean of total feed consumption of broiler chicks at the end of 4^{th} week in the dietary group T_0, T_1, T_2, T_3 and T_4 were 2549.58±1.00, 2550.00±0.30, 2589.95±0.50, 2578.87±0.80 and 2678.95±0.50 respectively. The highest average feed consumption was found in T_4 (2678.95±0.50) and lowest result was in

T_0 (2549.58±1.00) group. Garlic extract (2.25 mL/kg of feed) stimulated chicken's appetite, outcome in higher feed intake and thereby higher body weight gains (Brzoska et al., 2015). Hernandez et al. (2004) reported no difference in the feed intake or FCR in broilers fed 200 mg/kg of diet with essential oils extracted from oregano, cinnamon and pepper or 5000 mg/kg of diet with labiates extract from sage, thyme and rosemary. Windisch et al. (2008) reported that improved feed intake and digestive secretions are also observed in animals given phytobiotic-supplemented feed. Though the feed consumption increases with the addition of cinnamon and garlic in feed but there was no significant difference among the treatments.

Feed conversion ratio (FCR)

Data presented in the Table 4 showed that FCR of broiler chicken was not significant (P>0.05) among the treatment groups. However, FCR in the dietary group T_0, T_1, T_2, T_3 and T_4 were 1.34, 1.34, 1.33, 1.33, and 1.31 respectively. It was found that antibiotics, herbs and phytogenic products could control and restrict the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in chicks' gut. This may lead to greater efficiency in the utilization of food, resulting in strengthen growth and improved feed efficiency (Bedford, 2000). In the current study, the positive impact of the additives on the digestive system and nutrient absorption is more distinct at younger ages, since the improved FCR seen in all supplemented groups at 28 day period was not reflected at slaughter age possibly due to the facts that older birds' nutrient requirements decrease with age. Cinnamon components (cinnamaldehyde and eugenol) have antibacterial and antiviral activity against a wide range of pathogens (Chang et al., 2001). The selective inhibition by cinnamaldehyde of pathogenic intestinal bacteria could have had a pharmacological role in balancing the intestinal microbiota in chicks resulting in better-quality productive traits. Jamroz and Kamel (2002) suggested that broilers fed with a combination of essential oils like capsaicin, carvacrol and cinnamaldehyde showed higher weight gain and better FCR. In this study, better FCR is obtained may be due to the better intensive management, lower mortality rate and the positive effect of cinnamon and garlic in the ration. Therefore, the cinnamon, garlic and the combined group of cinnamon & garlic showed better FCR than the control and antibiotic group.

Dressing percentage (DP)

In this study, the DP of dietary treatment groups T_0, T_1, T_2, T_3 and T_4 were 72.57±0.20, 74.87±0.10, 75.95±0.06, 75.99±0.32 and 76.21±0.24 respectively. However, the T_4 (0.2% cinnamon + 0.2% garlic) group showed better DP than the T_0 (control) and other groups. Al-Kassie (2009) found that different levels of oil extract obtained from thyme and cinnamon had significant effects on DP, abdominal fat, and internal organs percentage (liver, heart and gizzard). Therefore, the addition of cinnamon and garlic in broiler diet can improve the DP of commercial broiler.

Survivability

The survivability rate is presented in Table 4. Survivability rate was higher for the control, cinnamon, garlic and the combined cinnamon and garlic treated group (100±0.00) than the antibiotic group (95.56±0.50) but there was no significant (P>0.05) difference amongst them.

The overall survivability (0-4 weeks) during the experimental period was higher in the treatment groups. This result is similar to the result Valavi et al. (2015) who reported that supplementation of diets with garlic and cinnamon powder may improve antioxidant system and some blood parameters in broilers showed to heat stress. Therefore, the possible cause of survivability in this study might be due to the development of immunity amongst the treatment groups than the control.

Carcass characteristics

Carcass weight

Data presented in Table 5 displayed that the carcass weight in the different treatment groups is better than the control and antibiotic groups. The results discovered that the treatments had significant effects ($P<0.05$) on dressed breast, back, and wing in the T_4 treatment than the other treatments. However, there is no significant difference in thigh and drumstick within the treatment groups. However, in the treatment T_4 group (0.2% cinnamon + 0.2% garlic) the carcass weight is better than in other treatment groups. Javandel et al. (2008) and Onibi et al. (2009) found that garlic enhancement had no important impact on the main components of the carcass and the structures of the liver. Raeesi et al. (2010) suggested that between various treatments, enrichment of 1.0% and 3.0% garlic in the broiler diet had no major influence on the relative weights of carcasses, fat pads, or digestive organs. Garcia et al. (2007) perceived that a blend of oregano, cinnamon and pepper oil (200 ppm) had no influence on carcass weight of broilers. However, breast weight (% of carcass) appeared to increase after the incorporation of a plant extract based on a blend of clove and cinnamon oil (100 ppm). Koochaksaraie et al. (2011) discovered that supplementation of cinnamon powder at the dose of 250 to 2000 mg/kg broiler diets did not have any effect on the carcass parameters. Therefore, the present study suggested that the addition of cinnamon and garlic in broiler diet separately and combined improve the carcass characteristics in broiler.

Relative weight of internal organs

Data presented in Table 6 showed the relative weight of internal organs (liver, heart, neck, gizzard, intestine, spleen and bursa) of broilers fed diet containing cinnamon, garlic, 0.2% cinnamon + 0.2% garlic and control and antibiotic added group. The results showed significant differences ($P<0.05$) among the different groups. The T_4 (0.2% cinnamon + 0.2% garlic), T_3 (0.2% garlic) and T_2 (0.2% cinnamon) treated group showed better result than the control group. It was also observed that there was no significant difference among the groups for the immune organ intestine, spleen and bursa but in all cases T_4 (0.2% cinnamon + 0.2% garlic) group showed better results than the others. Lee et al. (2003) discovered no significant differences in the internal organs of the broiler chickens when integrated with cinnamaldehyde (100 ppm). The Labiatae extract and the blend of carvacrol, cinnamaldehyde and capsaicin improved the digestibility of the feeds but no consequences were noted on organ weight (Hernandez et al., 2004). Feeding increasing levels of garlic up to 1.0% to male broilers for 7 weeks did not alter carcass yield and parts, organ weights, and intestinal mucous (Carrijo et al., 2005). Najafi and Taherpour (2014) stated no significant effect ($P>0.05$) on the relative weights of spleen, bursa of fabricius and thymus with dietary treatment of cinnamon. Therefore, in cinnamon and garlic

treated groups, the weight of internal organ is higher than in control group. This might be due to the positive effect of cinnamon and garlic on carcass trait of chicken.

Immune parameters

The immune parameter mainly WBC, Lymphocyte and Granulocyte was counted and the data has presented in Table 7. The WBC, Lymphocyte and Granulocyte were statistically significant (P>0.05) among different treatments. The highest granulocyte was in control (11.20±0.10) which indicates low immunity in control group. The lowest WBC (5.00±1.00), Lymphocyte (2.05±0.13) and Granulocyte (3.50±0.10) found in T_4 (0.2% Cinnamon + 0.2% Garlic). Although reports on the effect of garlic and cinnamon on immune responses in broilers are scarce, our results have similarity to Jafari et al. (2008) who observed that the inclusion of 1.0 and 3.0% of garlic powder did not enhance the serological response of broilers to newcastle vaccine. Cinnamon has great potential as a natural alternative medicine for the cure and inhibition of many serious diseases (Alzheimer's Parkinson's and Diabetes) and for its anti-inflammatory and anti-proliferative activities. Hossain et al. (2014) recommended that 1.0% cinnamon powder had a significant impact to increase the antibody SP ratio (ratio of sample and positive control) for Newcastle disease and lower the blood glucose level. According to the present findings, it is recommended that the addition of cinnamon and garlic in broiler feed significantly increases the immunity in broiler.

CONCLUSION

The research was conducted to investigate the potentiality of cinnamon and garlic on growth performance, carcass traits and immune parameters of commercial broilers. It could be concluded that the addition of cinnamon and garlic performed positively more or similar to antibiotic separately and the significant performance was observed when the cinnamon and garlic are added combined on growth performance, carcass characteristics and immune parameters of broiler chickens than the control and antibiotic groups. Therefore, the study recommended that the addition of cinnamon or garlic individually and combinedly of 0.2% cinnamon and 0.2% garlic to feed as an alternative to antibiotics in broiler production can be applied to prevent the human health hazard.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Evaluation of reproductive performance of indigenous buffaloes at Sreemangal Upazila

L Asad[1*], AU Himel[2], ANMI Rahman[1] and BF Amy[1]

[1]Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
[2]MS Fellow, Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

[*]Correspondence: lamyea_sau@yahoo.com

ABSTRACT

The present study was conducted with a view to knowing the reproductive performance of indigenous buffaloes at Sreemangal Upazila under the Moulovibazar district. Data were collected on a regular basis in 2021 from thirty (30) families by written interview paper. Out of 30 families; 26 own 33 milch buffaloes, 17 dry buffaloes, 12 heifer calves, 7 bull calves, and 10 bullocks were counted for calculation. This study demonstrated that the distribution of buffalo populations was scattered and the average buffalo populations were 3.038 per house-holds. The evaluated reproductive performance of indigenous buffaloes found the age at puberty was 44.06 ± 2.13 months, lactation length 204.70±20.68 days, milk yield 1.88±0.702 liters/day, gestation period 307.00±2.11 days, body weight 27.26±1.55 kg/calf, age at first calving 53.84±1.48 months, calving interval 1.72±.548 years and the average post-partum heat period was 147.6±18.68 days. The study revealed that the reproductive performance of the buffaloes was varying among breeds, farms, locations, areas, seasons, and management in the study area. In a concrete manner, this study gives a complete scenario of the reproductive performance of indigenous buffaloes at Sreemangal Upazila under the Moulovibazar district of Bangladesh which is strictly controlled by genetic and non-genetic factors.

Keywords: Buffalo, Reproductive performance, Puberty, Gestation period

INTRODUCTION

Buffalo has a significant role in the agricultural economy of many developing countries by providing milk, meat, and draught power. The world population of buffalo is estimated to be 199 million (FAOSTAT, 2012) with more than 96% of the population located in Asia. In recent decades, buffalo farming has expanded widely in the Mediterranean and Latin America as well as, in Central or Northern Europe where several herds were introduced (Shamsuddin et al., 2001).

Dairy buffaloes have been used for milk production in India, Pakistan, Bangladesh and some other South Asian countries, the Middle East, and Italy; while dairy characteristics are being induced in the local population of Indo-Chinese Region and South America through crossbreeding with Pakistani Nili Ravi and Indian Murrah buffaloes. The milk yield was then increased from 700 to 2,000 kg/year (Ghaffaret al., 1991).

The buffalo can utilize poorer quality roughages, adapt to harsher environments, and are more resistant to several bovine tropical diseases (Warriach et al., 2015). Despite these merits, buffalo

have relatively poor reproductive efficiency irrespective of their location throughout the world. Buffalo exhibits many of the known reproductive disorders including delayed onset of puberty, poor oestrus expression, longer postpartum ovarian quiescence, and most importantly lowered conception rates, particularly when bred artificially (Gordon, 1996). However, higher fertility could be achieved through better feeding and management (Qureshi et al., 2010). It can be seen that there is limited quality research in the area of basic physiology, health, management, nutrition, and applied reproduction; because buffalo are located mostly in developing countries with meager resources (Qureshi et al., 2010).

We discuss the impact of the various techniques as well as bottlenecks and possible future development which will lead to improved reproductive performance in this species. Like other developing countries, animal production systems and their use vary widely in Bangladesh with the climatic condition, topography, and socio-economic condition. The production systems are characterized by the small number of animals with no or minimal inputs, low outputs, and periodic demolition of animals by disease and are mostly maintained under scavenging systems (Saadullah, 2012). Buffaloes are a better converter of poor-quality fibrous feeds into milk and meat (Habib et al., 2017). They are reported to have the capacity of digesting 5% more crude fiber than high-yielding cows; and 4-5% more efficient in utilizing metabolizable energy for milk production (Mudgal, 1999).Terramoccia et al., (2000) reported a better degradation rate of both crude protein (CP) and protein-free dry matter (DM) in buffaloes than in cattle. Growing buffaloes may utilize coarse feed more efficiently than cattle, have more disease resistance ability and produce more solids in milk (Dubey et al., 1997) and require fewer management inputs. It has also been reported that buffalo holds a strategic place in the overall livestock economy of Bangladesh and serves three important purposes such as milk, meat, and draught power supply (Ghaffar et al., 1991). With increasing population and economic stability, the demand for milk and milk products like butter cheese, ghee, yogurt, and ice cream has increased. During 2006-07, milk production in Bangladesh was 22.80 Lakh Metric tons whereas the current production is 72.75 Lakh Metric tons. The present demand for milk production in BD is 146.91 Lakh Metric tons and availability is 72.75 Lakh Metric tons hence the deficit is about 50.47%. Though milk production increased by 68.66% compared to the last 10 years but there are also 74.16 Lakh Metric tons, still we need to increase (50%) milk production in order to meet up the demand (DLS, 2016). In India, a white revolution occurred long ago through dairy buffalo rearing, and 67.99% of milk comes from buffalo whereas dairy buffalo production is very limited in Bangladesh as only 0.039% of milk is from buffalo. Major percentages (>90%) of milk is coming from dairy cows in Bangladesh. It is expected that buffalo will ultimately emerge as the future animal of dairy-cum-meat industry in the region (Dhanda, 2013). Therefore, we assume there is huge potential for dairy buffalo production in Bangladesh. That's why it is needed to gear up dairy buffalo production to enhance food security from livestock origin.

The dynamics of buffalo production systems in the South Asia region are transforming day by day due to increasing the population more rapidly specially in Asia for its emerging role in economic development (Dhanda, 2013). Saadullah (2012) observed that Buffalos production

system varies widely in accordance with climate, soil, and socio-economic opportunities in Bangladesh. Buffalos graze in a natural pasture in coastal areas. A total of 11, 5, and 84% of farmers reared buffalo for milk purposes, meat purposes, milk and meat purposes, respectively in coastal areas (Nahar, 2015). According to Saadullah (2012), buffaloes are kept mainly for specific purposes, i.e. either for milk or for meat production in Bangladesh under a semi-intensive system. It is an important livestock resource in several countries of South Asia and the Mediterranean regions. Buffaloes play a vital role in food security and poverty alleviation in South Asia, because of the largest population comprising diverse and the best buffalo germplasm in the world. Of the 194.29 million world buffalo population, 97% is concentrated in Asia and 57% in India alone (FAO, 2014).Due to high fat content of buffalo milk, it is the most preferred species and is called the Black gold of Pakistan (Bilal et al., 2006). The climatic condition of Bangladesh is nearly similar to India and has many rivers and marshy lands that favor raising buffaloes. Recently the Government of Bangladesh, the private sector, and research organizations have given emphasis on Buffalo production. The availability of milk in Bangladesh is only about 208.61 ml/day/head (DLS, 2021-22) whereas; the availability of milk in India and Pakistan is about 290 ml, and 525 ml/day/head respectively (Hamid et al., 2016). This figure indicates that Bangladesh needs to give more emphasis on milk production to fulfill the national demand. The indigenous dairy cows are low producers and the crossbred cow has limitations regarding disease resistance, repeat breeding etc.The use of other mammalian livestock species for milk production could help to improve the scenario (Siddiki, 2017) and the success of India and Pakistan dairy industries based on buffalo might be a good example for Bangladesh. However, buffaloes are low producers in Bangladesh, because of poor genetic potentialities, poor nutrition, longer puberty age, seasonality of breeding, longer calving interval, high calf mortality, and poor management practices (Nahar, 2015; Faruque and Amin, 1995; Shamsuddin et al., 2001). Faruque and Amin (1995) and Uddin et al., (2016) reported the reproduction and production performances of buffaloes in the Noakhali district of Bangladesh. However, there is no report on the reproductive performances of buffaloes in the Moulovibazar district of Bangladesh where a good number of buffaloes exist. Moreover, different management systems e.g. extensive and semi-intensive systems were followed in this area. Semi-intensive system was introduced very recently to emphasize lactating buffaloes. Therefore, the present study was designed to investigate the few reproductive characteristics of indigenous buffaloes in Bangladesh and hence find out the factors which control the reproductive performance of indigenous buffaloes at Sreemangal upazila.

MATERIALS AND METHODS

The present study was completed under the Department of Animal Nutrition, Genetics and Breeding, Sher-e Bangla Agricultural University (SAU), Dhaka-1207, with the financial support of the Sher-e Bangla Agricultural University Research System (SAURES).

Study area

This study was conducted at different places of Sreemangal Upazila. Three villages namely Poshchimbharaura, Nawagaon and Uttoruttorsor were purposely selected for the study. The

ecology of this area is suitable for buffalo rearing. The average temperature is 24.5℃ and the annual rainfall average is 2081 mm. Grazing land is available for buffalo. In this study, two approaches were adopted viz.

i) Initial in-depth monitoring of the activities of the household before collection of data.
ii) Recording reproductive performance directly from the farmers in their household.

Study period & data collection

The 26 families of buffalo owners were taken from Sreemangal Upazilla under the District of Moulovibazar for this study. Data for the experiment were conducted through door to door visits at farmer's houses during June/2021 to Dec/2021. Data was collected through a previously prepared interview schedule. Questionnaire includes both open and closed questions to collect data with view to objectives of this study. Direct interview method was used for collection of information.

Parameters of the study

To evaluate the reproductive performance of Buffaloes the following parameters were considered.

Age at puberty

Age at puberty is the time between birth and first estrus. When a female shows the sign of first estrous that age counts as the age of puberty. By observing the wagging tail, swelling, watery discharge from vulva, jumping tendency to others and bleating detect the age of puberty of Buffalo.

Lactation length

Lactation length means the period when a Milch buffalo gives milk. Lactation period of Buffalo is longer than other animals. The total period when Milch buffalo gives milk was recorded in a data sheet and analyzed.

Milk yield

Milk yield means milk production expressed in kg per animal per day. The milk yield of indigenous buffaloes was recorded in a data sheet and analyzed.

Gestation period

Pregnancy period is known as gestation period. During gestation period a fetus develops, beginning with fertilization and ending at birth. This period is the time of conception to parturition.

Birth weight

Weight at first calving means the body weight of the calf when giving birth first. The body weight of the calf was recorded in a data sheet for analysis.

Age at first calving

Age of first calving indicates the time when buffalo give first birth to a calf. It is the time of interval between birth and first calving. That is recorded in a data sheet for analysis.

Calving interval

The term calving interval refers to the period from one calving to the next calving. Data were recorded in data sheet for analysis

Post-partum heat period

Post-partum heat period means the first heat after calving. Data was recorded in a data sheet for analysis.

Statistical Analysis

The collected data of the experiment were compiled and included to the Excel spreadsheet for statistical analysis. The values of reproductive performances were analyzed by using Statistical Analysis Software (SAS, 1998).

RESULTS AND DISCUSSION

The finding is described in accordance with the objectives of our study. The results of this study for different traits are presented in different Tables. The number of buffaloes under the selected Sreemangal Upazila has been shown in Table 1.

Table 1 Average number of buffaloes per house-holds at Sreemangal Upazila under the district of Moulovibazar

Types of buffaloes	No. of buffaloes	No. of house holds	Total No. of buffaloes	Average
Milch	33	26	79	3.038
Dry	17			
Heifer calf	12			
Bull calf	7			
Bullock	10			

Table 2 Reproductive performance of indigenous buffalo at Sreemangal Upazilla under the district of Moulovibazar

Parameter	Types of Buffaloes	No. of Buffaloes	Mean ± SD
Age at puberty (Months)	Buffalo	79	44.06±2.13
Lactation Period (Days)	Milch buffalo	50	204.70±20.68
Milk Yield (Litre /Day)	Milch buffalo	50	1.88±0.702
Gestation period(Days)	Pregnant buffalo	50	307.00±6.06
Age at first calving(Month)	Buffalo	50	53.84±1.48
Birth Weight (kg)	Calf	19	27.26±1.55
Calving Interval(years)	Buffalo	50	1.72±.548
Post-partum heat period (Days)	Buffalo	50	147.6±18.68

Age at puberty

The average age at puberty of indigenous buffaloes at the studied area was 44.06 ± 2.13 months and this finding is agreed by Jainudeen (1984) who reported that the age at puberty of buffaloes were delayed (42-48 months). The recent study at Kawkhali upazila under pirojpur district was reported that age at puberty was 45.03±6.27 months (Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh 2016).The collected data of age at puberty of buffaloes are presented in Table 2. Perera, (2011) reported that the age at puberty is influenced by genotype, nutrition, management, climate, and under favorable conditions occurs at 15-18 months in river buffalo and 21-24 months in swamp buffalo. The ovaries of buffaloes are smaller than in cattle and contain fewer primordial follicles. Buffalo are capable of breeding throughout the year, but in many countries a seasonal pattern of ovarian activity occurs. This is attributed in tropical regions to changes in rainfall resulting in feed availability or to temperature stress resulting in elevated prolactin secretion, and in temperate regions to changes in photoperiod and melatonin secretion. The delayed age of puberty in this study also supports the previous statement.

Lactation length

The average lactation length of indigenous buffaloes at Sreemangal Upazilla of Moulovibazar district was 204.70±20.68 days where the findings of Faruque et al., (1990) studied that the lactation length of indigenous buffalo was 275 days. Faruque and Amin (1995) mentioned that the lactation length of indigenous buffaloes in the coastal area was 270 days. EI-Kirabi (1995) stated the lactation length of Egypt buffalo was 210 to 280 days. The recent study at Kawkhali upazila under Pirojpur district reported that the lactation period was 242.60±41.46 days. The average data of lactation period of indigenous buffaloes are presented in Table 2. The lactation length of the buffalo depends on several genetic and non-genetic factors. Maraiet al., (2009) found similar lactation lengths of the buffaloes found in this study and reported that lactation length is affected by the season of the birth and increased with the advancement of parity. In this study non-genetic factors, seasons, and parity might have the possibility to affect the lactation length of the buffaloes as well.

Milk yield

In the study area, the average milk yield of indigenous buffaloes was found 1.88±0.702 liters/day. Studies revealed that the daily milk production of Bangladeshi indigenous buffalo usually varied from 2.70-2.89 liters (Amin et al., 2015). However, comparatively higher milk production of 3.33-3.43 liters per day has also been reported in indigenous buffaloes by Karim et al., (2013). Afzal et al., (2007) reported that Milk production was lower in the first lactation than that in 2nd, 3rd and 4th lactations. Milk yield per lactation increased with increasing lactation length. The season of calving had a significant effect on milk yield. In this study, several factors might have affected milk production in the study area which corroborate the previous results found by different research findings.

Gestation period

The average gestation period of indigenous buffaloes of the studied area was 307.00±6.06 days. EI-Sheik and Mohamed (1967) found that the gestation period of Egyptian buffalo was

316.70±0.19 days.The recent study at Kawkhali upazila under Pirojpur district was reported that gestation period was 317.47±6.57 days.The average data of gestation period of indigenous buffaloes are presented in Table 2. It was reported that gestation length varies depending on breed. It was also found that in some cases the calving or parity number affects the length of gestation period of buffaloes (Anderson and Plum, 1965). It was also reported that the gestation length increases one to two days with each successive calf. Some investigators claim that the gestation length is influenced by the weight of the dam. Seasonal effects on gestation length have been found in a number of instances. In the present study the seasonal effects, dams weight, age, and parity number probably influenced the gestational period of the buffaloes at the study area.

Birth Weight

The average birth weight of indigenous buffaloes of the studied areas was 27.26±1.55 kg. In the present study, the birth weight of indigenous buffalo calves was more or less similar to the findings of Hussen (1990) who reported that the average birth weight of indigenous buffaloes was 26.74±2.4 kg in Tangail district. Faruque and Amin (1995) mentioned that the average birth weight of indigenous buffaloes of the coastal areas of Bangladesh was 22.00±3.50 kg which was almost similar to the findings of the present study, the average birth weight of 18 to 30 kg. The recent study at Kawkhali upazila under Pirojpur district reported that the birth weight was 23.77±5.20 kg. The average birth weights of indigenous buffaloes are presented in Table 2.Naqvi and Shami (1999) reported that higher birth weight was observed in male and female calves born to early maturing buffaloes as compared to late maturing group. It was observed that the birth weight of male calves increased non-significantly, whereas, birth weight of female calves increased significantly with the increase in parity. The present study revealed the calves' weight influenced by several factors from sire and dams individuals which is relevant with this study.

Age at first calving

The age at first calving of studied areas was 53.84±1.48 months which is more or less similar to findings of Fadzil (1969) who found that the minimum age at first calving was 3 years, 3 months and 26 days. Fadzil (1969) also found that the average age at first calving of rural Nili-Ravi buffaloes in Punjab was 45.84±0.19 months. The recent study at Kawkhali upazila under pirojpur district reported that age at first calving was 55.33±7.2 months. The average age at first calving of indigenous buffaloes are presented in Table 2.Bashyalet al., (2021) revealed that age at first calving depends on genetic factor (breed and heredity), physiological factor (age at maturity, fertility issues and anticipation of puberty), environmental factors (time of birth, season of breeding, location and photoperiod), health and nutritional factors and managemental factors (feeding practice and managemental system). During this study all the factors was not possible to consider at a time. Hence, the age at first calving found in this study possible to cover all the factors.

Calving interval

The average age of calving interval of indigenous buffalo was 1.72±0.548 years which are more

or less similar to the findings of Fadzil (1969) who found that calving interval was 639 days. Khan et al. (1990) reported that the calving interval of Nili-Ravi buffaloes in Pakistan averaged 552.44±18.4 days. The recent study at Kawkhali upazila under Pirojpur district reported that the calving interval was 650.93±79.57 days. The average age of calving interval of indigenous buffaloes are presented in Table 2. Cady et al., (1983) found that herd, year, season, and parity number also had significant effects on days open and calving interval. Month of calving was important for time until return to estrus. Herd and year influenced day's open, calving interval and services per conception whereas parity affected only days open and calving interval. Similar influence also occurs in the reproductive performance of buffaloes at present study.

Post-partum heat period

The average post-partum heat period of buffalo of the studied areas was 147.6±18.68 days. Ei-Sheikh and Mohammad (1967) found that the post-partum service interval of 1st, 2nd and 3rd calving for Egyptian buffalo were 192.95, 152.9 and 317.0 days respectively. Rao et al. (1973) described that the mean post-partum estrus was 146.2 days. Pervez et al. (1994) found the post-partum estrus interval averaged 171.79±4.01 days. The recent study at Kawkhali upazila under Pirojpur district reported that the post-partum heat period was 235.90±52.28. The average data of post-partum of indigenous buffaloes are presented in Table 2.It was explained that postpartum heat period is affected by several factors such as nutrition plane, milk yield, Body Condition Score (BCS) at calving, suckling, parity, calving season and other factors (Barile,Braille 2005; El-Wishy, 2007). In this study the variation of post-partum heat period is possibly due to the similar factors found previously.

CONCLUSION

The existing research showed a relatively overall reproductive performance of indigenous buffaloes in the Shreemangal upazilla at Moulvibazar district. Results obtained from this study have shown variations in reproductive performances of indigenous buffaloes. This study found that the reproductive performance of indigenous buffaloes depends on various factors such as management, breed, parity, seasons, feeding and environmental condition. Along with some genetic and non-genetic factors,the physiological and hormonal condition of the individual animals also affects the reproductive performances. Although all the factors have not been studied in this research, it assumes that a lot of factors control the performance in combination. From this study, it can be concluded that the buffalo owner needs to rear the animals in a scientific manner rather than traditional practices.In general, the adoptions of proper selection and appropriate breeding methods are essential for its genetic improvement to increase its productivity. The main problem in buffaloes are silent heat, if it can be managed properly, we can get more production to fulfill our demand. This research finds out some reproductive performance which helps for further research. If we provide better management, nutrition and AI (Artificial insemination) service for reproduction, we hope better production from buffaloes. Finally, these data will be helpful for future research and also give a guideline for buffalo development in Bangladesh.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Sensory and Physico-Chemical Quality Evaluation of Traditional, Improved and Commercially Dried *Puntius sophore*

MR Islam, MA Baten, AW Newaz*

Department of Fishing and Post Harvest Technology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

*Correspondence: awnewaz.fpht@sau.edu.bd

ABSTRACT

To evaluate the sensory and physical-chemical qualities of traditional, improved, and commercial dried punti (*Puntius sophore*), an experiment was conducted. Fresh punti fish was procured and dried for this purpose utilizing both traditional (TD) and improved methods (ID). Commercially dried (CD) punti fish was also procured from regional markets. The sensory evaluation of the improved dried products revealed that they were of higher grade than both commercial- and tradition-style dried punti fish. According to the findings, enhanced dried punti rehydrated more quickly than traditional and store-bought dry punti. The dried punti's moisture content ranged from 14.20.45% (commercial dried punti) to 22.50.8% (improved dried punti). When compared to traditional and commercial dried fish, improved dried fish had the highest levels of crude protein, crude fat, and ash. After 30 days of storage, market dried punti was found to be infested with insects. However, it was discovered that both traditional and improved dried punti remained pest-free for up to 120 days of storage. The results indicated that the improved drying techniques produced the best dried fish product in terms of sensory, physico-chemical, and food safety aspects.

Keywords: *Puntius sophore*, dried fish, sensory properties, proximate composition, water reconstitution, insect infestation.

INTRODUCTION

Fish has always been considered one of the most valuable animal protein sources for human. In addition, fish serves as excellent source of minerals, polyunsaturated fatty acids (PUFA) especially Eicosa Pentaenoic acid (EPA) and Docosa Hexaenoic Acid (DHA) (Goswami and Manna 2019). Fish is also considered as highly perishable food commodity owing to its spoilage prone biochemical constituents. Fish contain large number of fatty acids with high degree of unsaturation that are always susceptible to oxidation by atmospheric oxygen. This phenomenon frequently gives rise to rancidity with off-odor especially observed in smoked and dried fish (Cho et al., 1989). Therefore, in order to prevent the spoilage, fish needs quick preservation of fish which includes salting or brining, sun drying, solar drying, air drying and smoking in smoking kilns (Mustapha et al., 2014). Among these, drying is cheapest method of fish preservation and dried products are easily transportable, marketable and storable (Hassan et al. 2013; Ahmed et al., 2007). The dry fish considered as a good source of protein, vitamins and minerals in the diet of people in many parts of the world including Asia and Europe (Paul et al., 2018). The traditional sun-drying method usually takes 3-7 days to produce dried fish products (Islam, et al., 2013). Apart from nutritional value, dried fish has considerably longer shelf life and requires no specialized facilities for storage (Immaculate et al. 2013; Reza et al. 2005). In Bangladesh, there are wide variety of fish species are used to produce dried products mainly small indigenous species including mola, dhela, chapila, punti, chanda, and batashi etc.

(Majumdar et al 2017). Most of the people of Bangladesh who are linked with traditional fish drying are illiterate (Azam, 2002). In Bangladesh, traditionally fish are dried open unhygienic environment under without ensuring food safety aspects. The dried fishes are often subjected to the insect attack or microbial spoilage due and the quality is severely compromised due to non-conducive weather condition. Available reports from previously conducted study suggested that the quality of majority of the traditional sundried products available in the market are not up to the mark for human consumption and sometime they even possess serious health hazards (Paul et al., 2018). Furthermore, traditionally produced dried fish are frequently found contaminated with different kinds of banned detrimental pesticides which are lethal for human health (Bhuiyan et al., 2009). Therefore, to overcome these quality issues of dried fish products and for ensuring the production of safe wholesome dried product, different kinds of improved drying methods with different pre-treatments of fish have been established (Kumar et al., 2017 and Roy et al., 2014).

Punti, also known as pool barb, is a well-known member of the Cyprinidae family and a significant source of micronutrients (Goswami and Manna, 2019). It is sold widely and regarded in Bangladesh, India, Myanmar, China, Nepal, and Bhutan as being incredibly nourishing and delicious. In Bangladeshi local markets, this species is easily accessible in fresh, dried, and fermented forms. Punti that has been traditionally prepared and dried is sold in Bangladeshi markets. It is evident that basic sanitation and hygiene standards are not upheld during the drying of fish; as a result, insect infestation, the presence of dirt, filth, and pesticide residue are extremely prevalent issues with the dried fish products of Bangladesh (Nowsad, 2007). Farmers commonly employ drying to preserve fish as a least expensive option. Therefore, it is required to design a better drying procedure that includes pre-treating the raw material in order to increase the dried fish's quality. The impact of various drying techniques on the sensory and physico-chemical quality evaluation of dried punti remains unsettling in Bangladesh despite the development of multiple improved drying methods, including solar drying and ring tunnel dryers. Consumers can find the best dried fish by evaluating its nutritional content and shelf life as well as by comparing other dried items. The goal of the current study was to determine the sensory and physico-chemical characteristics of market-grade, enhanced, and traditional dried *Puntius sophore*.

MATERIALS AND METHODS

Sample collection

Punti (*P. sophore*) is a widely known small indigenous species (SIS) of Bangladesh. For experimental purpose specimen of this species were collected from rice field of Kustia district, Bangladesh by using traditional fishing gear (charo, bana, khunkhuni, thela jal).

Sample preparation

Traditional drying (TD)

The collected fish washed in an earthen pot locally called ‘Dhuksa’ in Kushtia region. For drying purpose scales were first removed and then fishes were gutted by stripping the belly open. Stripped fishes were then washed thoroughly with clean water for 3-4 times. Finally, fish

samples were dried under direct sunlight in bamboo made tray (‘Kula’) placed on the rooftop for 5-6 days.

Improve drying (ID)

In improved drying technique fish samples were gutted and washed properly. As a part of pre-treatment, samples were mixed with 4-5% salt and 2-3% turmeric powder. Samples were placed in ‘kula’ for drying. Kula was then covered with thin polythene sheet which helped increase the temperature and provided protection against insect infestation. Samples were dried properly under sunlight within 2-3 days.

Commercially dried (CD)

Commercially dried (CD) punti sample was collected from the local markets located in Mohammadpur and Kawran Bazaar.

Sensory analysis

The sensory parameters such as appearance, color, flavor and overall preference of traditional, improved and market dried products were observed and all the samples were unbiasedly encoded prior to sensory evaluation. Ten experienced members (aged between 20 to 45 years) were selected from Sher-e-Bangla Agricultural University. Dried fish samples were examined on the basis of Hadonic scale where 9 denotes ‘extremely liked’ and 0 denotes ‘extremely disliked’ (Roy et al., 2014).

Water Reconstitution Analysis

Percentage of water is absorbed by dried sample at a certain temperature and time is called water reconstitution. It is used to assess the physical parameters of dried product (Prodhan and Kamruzzaman, 2011). Each sample was weighed by electric balance and kept it in a conical flask. The flask is placed in the water bath for an hour at room temperature, 50°c and 60°c and weighed at every 15 min intervals for each temperature.

Water reconstitution was determined by the following formula (Haque et al., 2013)-

$$\% \text{ Water reconstitution} = (Wr - Wi) / Wi \times 100$$

Wi = Initial weight of the dry fish

Wr = Weight of dry fish after reconstitution,

Wr – Wi = Water reconstitution

Proximate composition

Protein, Lipid, Moisture, and Ash contents of dried punti fishes were determined by using standard AOAC method (1995).

Observation of insect infestation in stored condition

TD, ID and CD punti were stored in room temperature for four months. Samples were observed at 30 days interval and records were kept.

RESULTS AND DISCUSSION

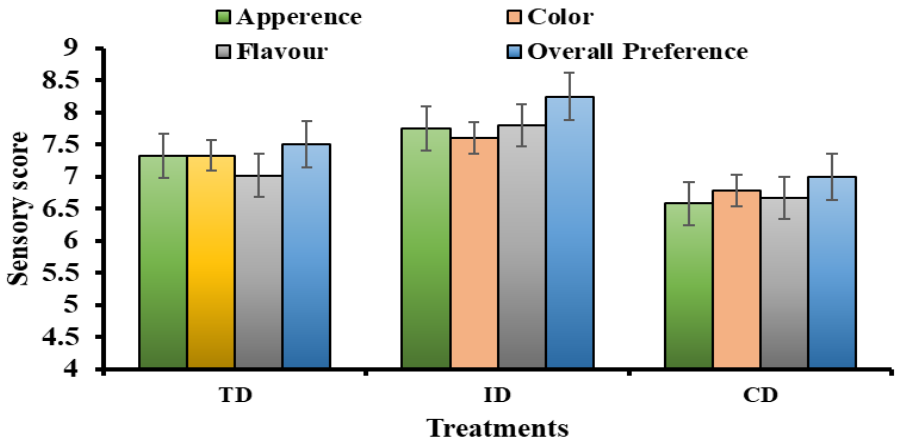

Fig. 1 Effect of the drying methods on the sensory properties of *P. sophore*

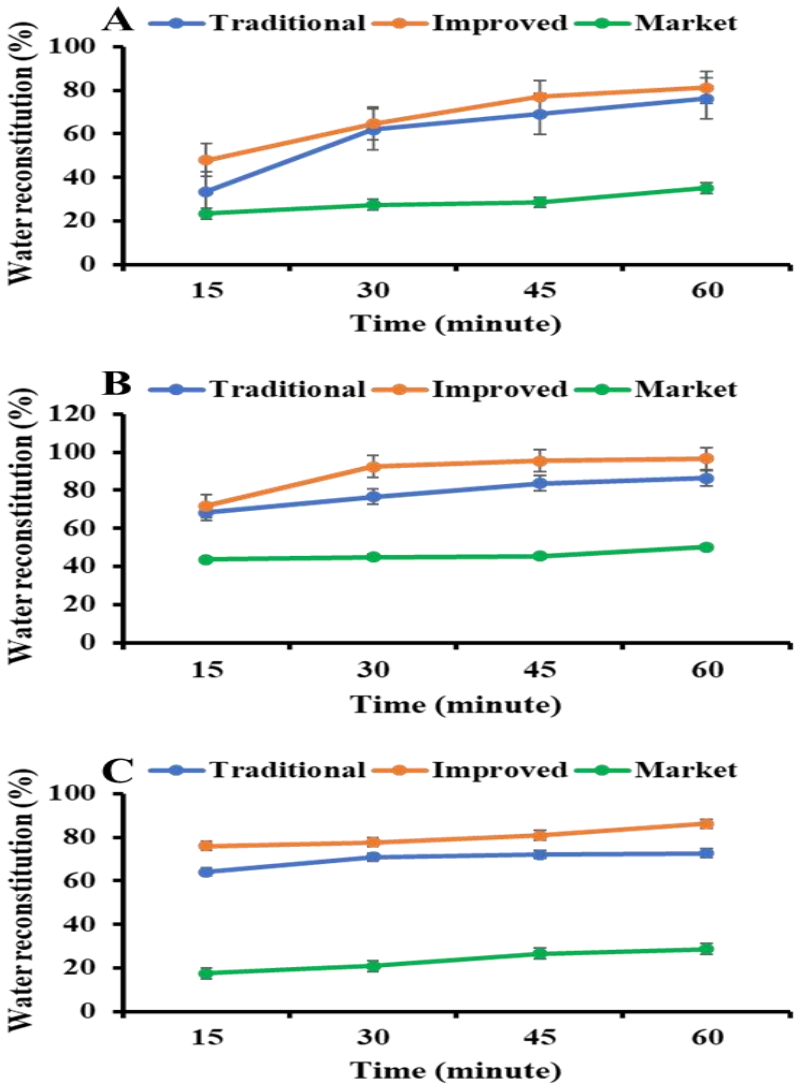

Fig. 2 Water reconstitution property of *P. sophore* at (A) room temperature, (B) 50 ºC and (C) 60 ºC for the different drying methods

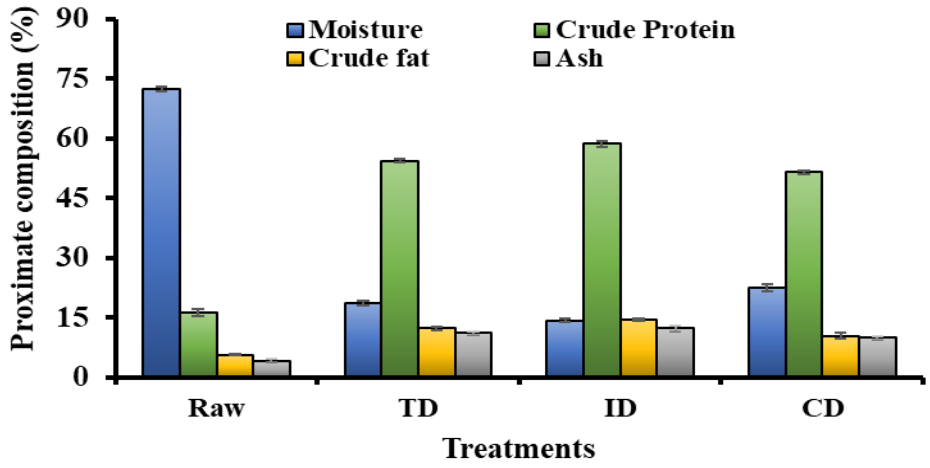

Fig. 3 Comparison of proximate compositions of fresh and dried *P. sophore* processed under different drying methods

Fig. 4 Observation of insect infestation in traditional dried-TD (A), improved dried-ID (B) and commercially dried-CD *P. sophore*

Sensory analysis of dried Punti fish

In case of improved dried punti (*P. sophore*), significantly the higher Hadonic scale values of were found for appearance, color, flavor and overall preference when compared to traditional and market dried Punti (Figure 1). The values recorded for traditionally dried method secured second position followed by commercially available dried punti collected from different market.

Water reconstitution property of sundried Punti

The results of the present study revealed that at ambient temperature (28-30°C), CD punti showed poor water reconstitution property with moisture content of 35.06% after 60 min of soaking. Whereas, in case of TD and ID dried punti comparatively higher water-holding capacity was observed after same duration of soaking with moisture content of 76.19% and 81.25%, respectively (Figure 2A). On the other hand, at 50°C and 60°C temperature CD, TD and ID punti showed a moisture content of 50.18%, 28.69%, 86.25% (Figure 2B) and 72.62%, 96.58% and 86.15% (Figure 2C) after 60 minutes soaking, respectively.

Proximate composition of sundried Punti

The moisture content of the dried *P. sophore* ranged from 22.5±0.8% to 14.2±0.45 on a wet matter basis where the moisture content of fresh fish was 72.33±0.5%. After completion of

drying, final moisture content of TD, CD and ID punti was recorded as 18.65±0.6%, 22.5±0.8% and 14.2±0.45%, respectively. Comparatively lower moisture content was found in the ID product in compare with the TD and CD. Following the similar trend in terms of quality, the highest protein content was observed for ID punti fish (58.55±0.67%) whereas the lowest value was found in the CD fish (51.45±0.55 %) collected from market. The lipid content of dried fish ranged between 14.4±0.33% to 10.43±0.66%, with highest value in ID fish product. The highest ash or mineral content of dried punti found in ID product (12.3±0.75%) and lowest in (9.87±0.57%) in CD punti fish. The average moisture, crude protein, crude fat and ash content of collected raw fresh punti specimens were 72.33±0.5% 16.25±0.77%, 5.68±0.23% and 4.1±0.44%, respectively (Figure 3).

Insect infestation

All three types of dried fish samples were packed in airtight zipper bag and stored in room temperature for 120 days. The study found insect infestation occurred in CD punti after 30 days of storage. On the other hand, any sort of insect infestation was apparently present in both TD and ID *P. sophore* within 120 days period of stored (Figure 4).

Dried fish is a highly popular and delectable cuisine in Bangladesh and other nations. The preparation of dried fish often involves a variety of drying techniques and pre-treatments. The biochemical, microbiological, and sensory characteristics of the dried fish are significantly influenced by these various techniques.

Using panel members' senses and the Hadonic scale approach, sensory analysis including appearance, color, flavor, and overall preference of dried fish were evaluated to estimate quality. The overall acceptance of the dried fish produced using the enhanced drying technique, the conventional drying technique, and the market dried punti, respectively, was found to be greater. It has been asserted that a dried fish's sensory score correlates with the quality of the product, and that the opposite is also true (Sultana et al., 2011; Roy et al. 2014). Market-available sun-dried fish had unappealing appearance, color, and flavor, whereas dried fish made using the solar tunnel drying process had significantly higher quality in all respects (Rahman et al 2012). Our findings suggested that compared to dried fish produced using the traditional approach and purchased from the market, fish produced using the improved drying process exhibited slightly higher quality.

The capacity of dried fish to hold water increases with increased soaking time and temperature (Akintunde et al., 2008). Overall, the fish that was dried using the improved approach rehydrated more quickly than fish that was dried using the conventional method or purchased at the market. According to some reports, warmer water can cause fish muscles to become more permeable, speeding up the process of water absorption (Akintunde et al., 2008). Reza et al. (2005) have noted that the physical characteristics of dried fish have a favorable impact on rehydration ability. Furthermore, in addition to time and temperature, the capacity of dried fish to absorb water depends on the species (Rasul et al., 2018).

With longer soaking times and warmer temperatures, dried fish may hold more water (Akintunde et al., 2008). Overall, compared to fish dried using the traditional method or fish bought at the market, fish dried using the improved method rehydrated more quickly. Some reports claim that fish muscles can become more porous in warmer water, accelerating the process of water absorption (Akintunde et al., 2008). According to Reza et al. (2005), dried fish's physical attributes have a positive influence on its capacity to rehydrate. The ability of dried fish to absorb water also depends on the diversity of species, in addition to time and temperature. When fish was treated with salt to help lower its water activity more quickly, the improved products showed relatively lower levels of moisture content. According to Hasan et al. (2006), some small indigenous species' conventional and solar-dried products had moisture contents that varied from 26.02% to 27.33% and 13.71% to 19.30%, respectively.

The protein content of the dried fish produced using conventional, improved drying methods, and market samples was 54.350.3%, 58.550.67%, and 51.450.55%, respectively. These figures were confirmed by Hasan et al. (2006), who discovered that the protein content of dried fish species ranged from 44.72% to 60.33%, and that the protein content of other small dried fish species also ranged from 44.72% to 60.33%. According to report of Azam et al. (2003) on the biochemical analysis of a few dried fish products, the protein content ranged from 40.69 to 66.52%. The dehydration of water molecules between the proteins, which led to the protein aggregation, increased the protein level in the dried fish (Nagwekar et al. 2016, Ullah et al., 2016).

The dried fish that was acquired from the market had the lowest amount of lipids, which may be because conventional drying procedures cause lipids to oxidize more quickly. According to reports, dried fish purchased from the market had a higher moisture content than dried fish produced using an improved drying technology, which had higher protein and fat values (Immaculate et al., 2012).

On the other hand, the lipid content of various dried fish species ranges from 14.40.33% to 10.430.66%, with improved dried fish having the highest and market dried fish having the lowest amounts. The results of Hasan et al. (2006), who found that the lipid content of dried fish species ranged from 8.91% to 18.07%, supported these numbers. These values fall within the range reported by Flowra and Tumpa (2012), who found dried fish samples with lipid contents ranging from 5.38% to 15.86%.

The ash percentage of several dried fish species ranged from 9.870.57% to 12.30.75%, with market dried fish having the lowest value and improved dried fish having the greatest value. The market sample of the improved dried fish included the largest level of ash, which may have been caused by contamination with sand and dirt during drying. This data is almost identical to that of Azam et al. (2003), who discovered that the ash percentage of dried fish ranged from 5.08 to 12.14%. Our findings concur with those of Nurullah et al. (2006), who reported that the ash percentage of various dried fish species ranges from 9% to 19.32%. But there was no significant difference observed in the protein, lipid, or ash content in the dried fish produced by the various drying methods on a dry matter basis.

In the case of an insect infestation, it was found that traditional and improved dried products performed better, however market goods performed poorly because of the insect infestation. According to a study, insect infestation in dried goods is a significant issue when drying fish (Flowra et al., 2013). Fish processors typically employ hazardous chemical pesticides without taking into account the deadly consequences of these pesticides on customer bodies to protect dried fish from insect infestation. The most typical insecticides used in dried fish to prevent insect infestation are DDT and Nogos (Nowsad, 2007).

CONCLUSION

The results of this study revealed that different drying methods with different pre-treatments have a significant role on the physico-chemical and sensory properties of the dried *P. sophore*. Sensory and physico-chemical characteristics were highly acceptable for the improved dried fish products, where fish were pre-treated with salt (4-5%) and turmeric (2-3%). The market dried fish were of comparatively poor quality in terms of proximate composition, sensory analysis and water rehydration when compared with the traditional and improved dried fish products. Our results indicate that the dried fish produced by the improved drying method showed comparatively better quality than the dried fish produced by the conventional drying methods.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interests.

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