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ABSTRACT

This study was conducted under the Department of Physiology and Pharmacology to determine the effects of Neem and Nishyinda leaves supplementation on growth performance and GUT biosis in broilers. A total of 40 day old broiler chicks were divided into four groups A, B, C and D. Group A was considered as control, fed only with commercial broiler ration. Group B supplemented with formulation of 2 gm grinded Neem leaves, Group C with 2 gm grinded Nishyinda leaves and Group D with 1 gm grinded Neem leaves plus 1 gm grinded Nishyinda leaves per liter of water respectively. Observations were recorded for live body weight, weight gain, feed consumption, feed efficiency, hemato-biochemical parameters and GUT biosis specially the parasite of birds in six weeks. Body weights were increased significantly ($p<0.05$) in all treated groups in respect to the control and highest was recorded in combine Neem plus Nishyinda supplemented groups (Group D). No significant ($p>0.05$) differences were observed among the groups for PCV and TEC values. Hb content increased and ESR decreased significantly ($p<0.05$) with combine Neem plus Nishyinda supplemented group (Group D). The serum SGOT and SGPT levels were also decreased with the same kind of supplementation. In treatment group there was significantly decreased of parasitic eggs, but incase of control group no decrease of parasitic eggs. There was no significant pathological change in any internal organs of the broiler of treated groups. Best result was found in the group D. The present study reveals that combine supplementation of Neem plus Nishyinda gives better result over other groups in respect to body weight gain, feed conversion ratio, hemato-biochemical parameter and profitability without making any health hazard of the broilers.

EFFECTS OF NEEM AND NISHYINDA LEAVES SUPPLEMENTATION ON GROWTH PERFORMANCE AND GUT BIOSIS IN BROILER

A Thesis

By

ATIKUR RAHAMAN
Registration No. 1305105
Session: 2013-14
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MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
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LIST OF ABBREVIATIONS

B.wt.	:	Body weight
Conc.	:	Concentration
Cu. mm	:	Cubic millimeter
d.w.	:	Drinking water
ESR	:	Erythrocyte Sedimentation Rate
et al.	:	Associates
Fig.	:	Figure
gm	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit	:	Liter
Ltd.	:	Limited
mg	:	Milligram
mm^3	:	cubic millimeter
No.	:	Number
PBS	:	Phosphate Buffer Solution
PCV	:	Packed Cell Volume
PM	:	Population Mean
SE	:	Standard Error
SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
0^0C	:	Degree centigrade

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CHAPTER 1
INTRODUCTION

Bangladesh is an agricultural country. Bangladesh is also a highly populated country. Large amount of people depend on agriculture for their livelihood. Poultry sector is one of the branches of agriculture. Poultry production especially

chickens and ducks has attained an important place in agricultural economy of Bangladesh both through contribution to GDP and employment especially in urban areas. About 80% of the total population of 160 million is living in the 68,000 villages of Bangladesh and almost each and every village home holds 6 to 7 chickens (Samad *et al.,* 2005). From poultry people get egg, meat which fulfills the protein demand of huge amount of people of Bangladesh. There are so many sources of protein but it is not possible to fulfill the demand without broiler. Because the duration of broiler rearing is very short and within 36 - 42 days it is ready for marketing and suitable for human consumption. It also brings very short time return to farmer. According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for broilers production. There are various Bacterial, Viral, Mycoplasmal, Fungal and Parasitic disease occurs in broiler, which increase the mortality rate, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotics as a growth promoter or life savings for broiler. As a result, each and every broiler is a depot of antibiotics. When these broilers are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues (Kibria *et al.,* 2009). With the development and wide use of synthetic and semi-synthetic antibiotics, pros and cons have been experienced throughout the bacitracin, lincomycin, penicillin, chlortetracycline and virginiamycin promote growth because of an affects on the microflora in the gastrointestinal tract. Antimicrobial resistance in zoonotic enteropathogens including *Salmonella*, *Escherichia coli* (*E. coli*), 50 years, which have been directed research back to natural antimicrobial products as indispensable resources. Consequently there is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding. Antibiotics such as and *Enterococci* in food animals is of special concern to human health because these bacteria are likely to transfer from the food chain to humans. So, scientists are again concentrating on the use of our

ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the production. Such plants, Neem (*Azadirachta indica*) and Nishyinda (*Vitex nigundo*) are indigenous plant of Asian subcontinent known for its useful medicinal properties since ancient times. Neem and Nishyinda has attracted worldwide prominence due to its vast range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepato-protective and various other properties without showing any adverse affects (Greathead *et al*., 2003, Chowdhury *et al*., 2009). Also, Neem and Nishyinda promote growth and feed efficiency of birds, because of their antibacterial and hepatoprotective properties (Singh *et al*., 1996). The Neem tree *Azadirachta indica* from the family Meliaceae (Von Rai *et al*., 1997) contains azadirachtin- a biologically active compound found in its seeds, bark and leaves (Wikipedia, 2007; Makeri *et al*., 2007) which is responsible for its varied medicinal uses (Schmutterer *et al*., 1990). Nishyinda (*Vitex negundo)* is a shrub that grows in the Philippines. The leaves of *V. negundo* possess discutients properties and are applied to rheumatic swellings of the joints. They may be applied locally to swellings from rheumatic arthritis. The juice of the leaves is used for the treatment of fetid discharges. The principal constituents the leaf juice are casticin, isoorientin, chrysophenol D, luteolin, p– hydroxybenzoic acid and D-fructose. Neem (*Azadirachia indica*) and Nishyinda (*Vitex nigundo*) dry leaves extract as medical herbs could be beneficial in immunosuppressant diseases of poultry. Neem and Nishyinda leaves contain a vast array of hemically diverse and iologically active ingredients (Dorababu *et al*., 2006). Low dose of Neem leaves extract have an inhibitory action on wide spectrum of microorganisms (Talwar *et al*., 1997) and immune modulator actions that induce cellular immune reaction (Devakumar and Sukt *et al*., 1993). Also, Craig *et al*., 1999 stated that several herbs could help providing some protection against bacteria and stimulate the immune system. By realizing all sorts of problem we are planning to rear broiler by using herbal medication instead of any antibiotics, to avoid human health hazards as well as economic broiler production to sustain small scale broiler farming in Bangladesh.

My study was performed to observe the following specific objectives:

(i) To observe the growth performance and profit of broilers by grinded plant leaves supplementation.
(ii) To evaluate hemato-biochemical parameter in relation to that supplementation.
(iii) To observe the GUT biotic environment by observing parasites of broilers.

CHAPTER 2

REVIEW OF LITERATURE

Neem, Nishyinda, Broilers and Weight gain:

The purpose of this chapter is to provide a selective review of the research works accomplished in relation to the present study. Literature on growth performance of

broiler supplemented with Neem and Nishyinda leaves related to this study has been reviewed under the following headings.

Ban on feed antibiotics:

Antibacterial substances are used in considerable amounts as growth promoters in animal husbandry, and carry incalculable risks for human health resulting from the use of particular feed additives (Witte *et al*., 2000). The indiscriminate use of antibiotics as feed additives could lead to an increased number of antimicrobial-resistant bacteria, and ultimately compromise the treatment of bacterial infections in humans (Gersema and Helling *et al*., 1986; Mcdermott *et al*., 2002). Many countries concerned about this problem have restricted and or banned the use of antimicrobial compounds in feed for food animals to slow the development of resistance, and some groups advocate similar types of measures in the United States (McEwen and Fedorka-Cray *et al*., 2002). Major changes occurred in the use of antimicrobial agents for growth promotion during the last 6 years in different countries. In 1986, the Swedish Government banned the use of antimicrobial growth promoters (Wierup *et al*., 2001). Denmark banned the use of avoparcin in 1995 and virginiamycin in 1998. The glycopeptide-resistant *E. faecium* in broilers was decreased after the ban of avoparcin from 72.7% in 1995 to 5.8% in 2000 (Aarestrup and Jensen *et al*., 2001).

Alternatives to feed antibiotic growth promoters:

There are a number of non-therapeutic alternatives to antibiotic growth promoters, including enzymes, (in) organic acids, probiotics, prebiotics, herbs, immune stimulants and specific management practices (McEwen and Fedorka-Cray *et al*., 2002. Acids control *in vitro* and *in vivo* growth of microbial flora. Prebiotics are 'non-digestible feed ingredients' which exert some selective effects on the intestinal microflora. The use of herbs and essential oils may relate to their antimicrobial activity against pathogenic bacteria and parasites (Banerjee *et al*., 1998).

Medicinal plants (herbs):

Ancient use of medicinal herbs:

Culinary herbs and their essential oils have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties (Suppakul *et al*., 2003). However, secondary plant metabolites are largely unexploited in 'conventional' animal production systems. In the past, plant metabolites were generally considered as a source of anti-nutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead *et al*., 2003). In contrast to their regulated status in India, China, and other countries, herbal medicines are regarded as dietary supplements for humans in the US and are widely used. It is reported that approximately one quarter of adults used herbs to treat a medical illness within the past year in the US. Herbs contain some complicated mixtures of organic chemicals that may vary depending upon many factors related to the growth, production, and processing of the herbal product. Though herbs with antimicrobial properties are reported, their use in broiler diets has not been studied extensively.

Biu *et al.*, 2009 showed that The hematological effect of aqueous extract of *Azadirachta indica* administration intra-peritoneally to chickens for 18 days with graded doses of 500, 1000 and 2000 mg/kg body weight was evaluated. Twenty (20) chickens weighing between 400 and 725 grams divided into four groups of five birds each were used. There was a significant decrease ($p<0.05$) in the mean packed cell volume (PCV%) and hemoglobin concentration (Hb) which were dose dependent, while the mean white blood cell count (WBC) did not alter significantly ($p>0.05$). The mean differential counts of the extract-exposed chickens indicated that the mean values for monocytes, basophils and eosinophils increased significantly ($p<0.05$) with increasing dose, while mean values for lymphocytes and heterophils decreased significantly ($p<0.05$). The significance of these findings is discussed.

Deshpande *et al.*, 2009 Experiments were conducted on layers from 24 weeks old to the age of 32 weeks to investigate the effect of Tulsi (*Ocimum sanctum*) on the performance of layers. 45 experimental pullets were randomly divided into three experimental groups of 15 pullets in each. Control (T_0) received standard layer diet, group T_1 received standard layer diet with Tulsi (0.5%), group T_2 received standard layer diet with Tulsi (1%). Supplementation of Tulsi leaf powder at 0.5% or 1% in layer diet did not affect body weight, egg production, egg weight, feed consumption and feed efficiency. The average egg yolk cholesterol and serum cholesterol was reduced significantly from 45th day onward in group T_2. The average serum HDL cholesterol was increased significantly on 60th day in group T_2. The average serum triglycerides were numerically reduced on 60th day in group T_2 followed by group T_1 The average serum LDL cholesterol was reduced significantly on day 60th in group T_2 followed by group T_1. As the effect of Tulsi leaves was gradual, long term feeding of it in laying hens diet at the rate of 1% of the diet, may be helpful in lowering egg and blood cholesterol.

Mahmood *et al.*, 2009 studied that The present work was aimed at knowing the effect of various levels of garlic (*Allium sativum*) and kalongi (*Nigella sativa*) as herbal growth promoters on the (i) growth performance of broilers and on the (ii) dressing percentage, relative weight of heart, gizzard, liver, spleen and pancreas of the broilers. One hundred and fifty day old broiler (Hubbard) chicks were divided in five groups viz., A, B, C, D and E. Group A served as control and was fed ration without any supplementation. Whereas group B and C were fed ration supplemented with 0.5% and 1.0% kalongi, respectively. Similarly the birds in-group D and E were fed ration supplemented with 0.5% and 1.0% garlic, respectively. The experimental rations consisted of broiler starter mash and broiler finisher mash, which were fed from 2-4 and 5-6 weeks of age, respectively. The supplementation of kalongi and garlic in the broiler ration significantly ($P<0.05$) improved the weight gain of the birds of various groups as compared to those of control group. The birds' (in group D) using ration supplemented with 0.5% garlic

gained the highest live weight (1588 g) among the treated groups and the best-feed conversion ratio (1.91). Different levels of the herbal growth promoters did not exhibit any significant influence upon the feed intake values of the experimental groups. There was no difference (P>.05) between the average dressing percentages, relative giblet weight (heart, gizzard, liver & spleen) and relative pancreas weight of the broilers fed rations with or without supplementation of garlic or kalongi. It is therefore concluded that dietary inclusion of garlic or kalongi in the rations may be used for economical and efficient production of broilers.

Tabassum *et al.*, 2009 studied that Effect of restraint stress on brain oxidative stress parameters and their modulation by *Ocimum sanctum* Linn (OS) were evaluated in male albino rats. Rats were subjected to restraint / immobilization stress 3h/day for 6 consecutive days. Post administration of aqueous extract of OS (100 mg/kg for 6 consecutive days) was given following restraint stress. MDA a marker of lipid peroxidation, nucleic acids and proteins were estimated in cerebrum, cerebellum and brain stem. Exposure to restraint stress caused a significant elevation in the rate of lipid peroxidation, reduction in nucleic acids and proteins as compared to control in all three regions of brain of male albino rats. Post treatment of aqueous extract of OS prevented the stress induced changes in these biochemical parameters. The results of the study indicate the protective nature of OS on different regions of brain against the detrimental effect of restraint stress.

Vara Prasad Reddy *et al.*, 2009 conducted in broiler chickens to evaluate the effect of dietary supplementation of Tulsi (Ocimum sanctum) and selenium on antioxidative enzyme levels. Total forty-two broiler chicks of day-old divided into six groups of seven each were used for this study. Ocimum sanctum leaf powder (0.25% and 0.5%), organic selenium (0.3 ppm) and their combinations were added to the basal diet. Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and Catalase levels in plasma were measured at the end of 3rd and 6th

week of age. Dietary selenium (0.3 ppm) supplementation in itself significantly (P<0.01) increased GSH-Px activity and supplementation of only Ocimum sanctum leaf powder (0.5%) significantly (P<0.01) increased SOD and Catalase levels. However, Ocimum sanctum leaf powder (0.5%) and its combination with selenium (0.3 ppm) more effectively enhanced the levels of SOD, GSH-Px and Catalase. It is concluded that dietary supplementation of Ocimum sanctum at 0.5% level and its combination with selenium (0.3 ppm) can combat oxidative stress in broilers there by increasing the anti-oxidative enzyme levels.

Wankar *et al.*, 2009 studied that A experiment was conducted on 120 day old broiler chicks divided into four groups, T0, T1, T2 and T3 which were supplemented with Neem leaf powder @ 0gm, 1gm, 2gm and 3gm/kg of broiler ration, respectively. Weekly observations were recorded for live body weight, weekly gain in weight, weekly feed consumption and feed efficiency of birds for six weeks. All the treatment groups T1 (813.03), T2 (855.07) and T3 (834.21) recorded significantly (P<0.01) higher means for live body weight than that of control T0 (768.69) group. All the treatment groups showed non-significant increase in weekly gain in weight, feed consumption and feed efficiency as compared to that of control group.

Ansari *et al.*, 2008 was conducted to determine the comparative efficacy of six medicinal plants including *Nigella sativa, Boerhavia diffusa, Withania somnifera, Ipomea digitata, Azadirachta indica and Corylus avellena* @ 4 g/kg of feed as growth promoter and their subsequent influence on the performance of broilers. 210-day-old chicks were randomly divided into 21 experimental units of 10 chicks each. These experimental units were randomly allotted to 7 treatments comprising of 3 replicates each. Commercially formulated broiler starter and finisher rations were offered *ad libitum* from 0-4 and 4-6 weeks of age. Authenticated samples of the plant materials were dried in shade, pulverized and mixed each @ 4g kg-1 of feed and offered to the chicks of the respective

treatment groups. Maximum gain in weight was observed with the *Withania somnifera* (1.819 kg) followed by *Nigella sativa* (1.805 kg) and *Azadirachta indica* (1.800 kg). The best cumulative FCR at the end of 6th week of age was for that of *Withania somnifera* (2.038) followed by *Nigella sativa* (2.054) and *Azadirachta indica* (2.083). The lowest results as regards FCR were recorded for *Ipomea digitata* (2.394) and *Boerhavia diffusa* (2.396). The results of the *Corylus avellena* (2.209) and control (2.235) were statistically similar. The maximum profit per bird was obtained from *Azadirachta indica* treated birds followed by *Nigella sativa* and *Withania somnifera* treated chickens as compared to control. It was concluded from this study that medicinal plants especially *Withania somnifera, Nigella sativa* and *Azadirachta indica* can be used as growth promoters in the poultry diets with better production performance.

Baskaran *et al.,* 2008 was conducted *Ocimum sanctum,* commonly known as 'Sacred basil' or 'Holy basil', is grown as a household plant in India. This preliminary phytochemical study was carried out in acetone, benzene and chloroform extracts and the results showed the presence of numerous phytochemical compounds. The antibacterial activity was analyzed using four different bacterial strains (*E.coli, Bacillus subtilis,* S*taphylococcus aureus* and *Klebsiella pneumonia*) by using agar disc diffusion method. Our bacterial assay revealed that the extracts showed good antibacterial activity, but the acetone extract didn't show any specific activity. The presence of the phytochemicals signifies the potential of *Ocimum sanctum* as a source of therapeutic agents and may provide leads in the ongoing search for antimicrobial agent from plants.

Mohan *et al.,* 2008 studied that a cost-benefit analysis was made of the effect of three organic growth-promoters on yield and quality of two vegetable crops, brinjal (*Solanum melonogena)* and tomato *(Lycopersicon esculentum*), grown under field conditions. Traditional Ayurvedic growth-promoters, Panchagavya and Amrit Pani, were compared with Bokashi made using Effective

Microorganisms (EM) technology. The results indicate higher yield and lower glycoalkaloid content in Bokashi-treated crops, followed by Panchagavya. Panchagavya was the most cost-effective growth-promoter followed by Amrit Pani and then Bokashi. We recommend the use of Panchagavya as an organic growth-promoter for small and marginally profitable vegetable-crop farmers.

Shaba *et al.*, 2008 estimated that 50 million cattles are at risk of being infected with trypanosomes leading to more than 3 million livestock deaths yearly. Human African trypanosomiasis is caused by *T. brucei, T. rhodesiense* and other species and transmitted by tsetse flies. It is estimated that currently about 300-500,000 people are infected with 50,000 deaths annually (1). Chemotherapy of trypamosomiasis is faced with problems such as limited choice of trypanocides in the market, high cost, toxicity, and emergence of drug-resistant trypanosome strains. Recent ethnopharmacology and ethnomedicine revealed that several medicinal plants possess trypanocidal compounds, which may hold the key for a future potential trypanocides (2, 3). Thus, new approach and trypanocides are highly needed to combat trypanosomes. Based on this, *V. negundo* leaves extract *was* screened for possible antitrypanosomal activity. This plant is found throughout greater part of India in warmer zones. Almost all its parts can be used medicinally for rheumatism, fever, diarrhea dyspepsia, anthelmintic and other diseases Different parts of the shrub has been used in veterinary practice in such conditions as Rinderpest, dropsy, paralysis, impaction and ephemeral fever (4). Varied chemical constituents such as alkaloid-nishindine; flavonoids-5-hydroxy-3,6,7,3,4-pentameth-oxyflavone and casticin irridoid glycosides 2 pentacyclic triterpenoids, betulinic acid (3 -hydroxylup-20-en-28-oic acid) and ursolic acid (2 -hydroxyurs-12-en-28-oic acid) were isolated for the first time from leaves of *V.negundo*. Pharmacological activities such as analgesic, antiinflammatory, antioxidant and insecticidal/pesticidal properties) and toxicity effects of *V. negundo*, had been identified.

Dorababu *et al.*, 2006 showed that standardized aqueous extract of Neem (Azadirachta indica) leaves (AIE) has been reported to show both ulcer protective and ulcer healing effects in normal as well as in diabetic rats. To study the mechanism of its ulcer protective/healing actions, effects of AIE (500 mg/ kg) was studied on various parameters of offensive acid-pepsin secretion in 4 hr pylorus ligation, pentagastrin (PENTA, 5 microg/kg/hr)-stimulated acid secretion and gastric mucosal proton pump activity and defensive mucin secretion including life span of gastric mucosal cells in rats.AIE was found to inhibit acid-pepsin secretion in 4 hr pylorus ligated rats. Continuous infusion of PENTA significantly increased the acid secretion after 30 to 180 min or in the total 3 hr acid secretion in rat stomach perfusate while, AIE pretreatment significantly decreased them. AIE inhibited the rat gastric mucosal proton pump activity and the effect was comparable with that of omeprazole (OMZ). Further, AIE did not show any effect on mucin secretion though it enhanced life span of mucosal cells as evidenced by a decrease in cell shedding in the gastric juice. Thus, our present data suggest that the ulcer protective activity of AIE may be due to its anti-secretary and proton pump inhibitory activity rather than on defensive mucin secretion. Further, acute as well as sub acute toxicity studies have indicated no mortality with 2.5 g/kg dose of AIE in mice and no significant alterations in body or tissues weight, food and water intake, hematological profile and various liver and kidney function tests in rats when treated for 28 days with 1 g/kg dose of AIE.

Halim *et al.*, 2006 carried out to see the effect of the aqueous extract of Ocimum sanctum Linn (Tulsi) with Vitamin E on biochemical parameters and retinopathy in the streptozotocin-induced diabetic albino male rats.Adult albino male rats weighing 150-200gm were made diabetic by intraperitoneal injection of streptozotocin in the dose 60 mg/kg in citrate buffer (pH 6.3). The diabetic animals were left for one month to develop Retinopathy. Biochemical parameters like plasma glucose, oral glucose tolerance and glycosylated hemoglobin HbA1c, were measured along with lipid profile, and enzymes like glutathione peroxidase (GPX), lipid peroxidase (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione -S- transferase (GST) in normal, untreated diabetic rats and diabetic

rats treated with Ocimum sanctum L extracts and vitamin E. Fluorescein angiography test was done for assessing retinopathy. Results on biochemical parameters were analyzed statistically by using ANOVA followed by Dunnet's 't'- test. A p-value of < 0.05 was considered as significant. Evaluation of biochemical profile in treated groups showed statistically significant reduction in plasma levels of glucose, HbA1c, lipid profile and LPO, and elevation of GPX, SOD, CAT and GST. Treatment of the diabetic animals with Ocimum sanctum and Vitamin E, alone and in combination for 16 weeks showed reversal of most of the parameters studied including plasma glucose levels. Angiography showed improvement in retinal changes following combined antidiabetic treatment.

Habib *et al.*, 2005 showed that a research work of herbal medicine viz. Neem leaf extract (*Azadirachta indica*), nayantara leaf extract (*Catharanthus roseus)* and bitter melon fruit (*Momordica charantia*) juice with the patent drug gliclazide (Comprid®, Square Pharmaceuticals Bangladesh Ltd.) were studied on blood glucose level, hematological parameters and on body weight in rats. Twenty-five apparently healthy adult rats were randomly divided into 5 equal groups namely A, B, C, D and E. One group (group A) was kept as control. The rest four groups (B, C, D and E) of rats were treated with gliclazide (Comprid®) @ 4.5 mg/kg bd. wt./day, neem leaf extract (NLE) @ 500 mg/kg bd. wt/day, nayantara leave extract @ 500mg/kg bd. wt./day and bitter melon fruit juice @ 500 mg/kg b. wt./day respectively for 14 consecutive days. Blood glucose levels were significantly ($P<0.01$) reduced in all treated four groups of rats (39.78-44.31%) in comparison to their pre-treatment values. Total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were not changed significantly in any treated group (B, C, D & E). Eosinophil and monocytes and hemoglobin contents were decreased significantly ($P<0.01$) in all groups. Among the herbal drugs used in the study bitter melon fruit juice was more effective (7.45%) in increasing the body weight in comparison with other herbal preparations i.e. NtLE (7.4%) and NLE (4.86%). From the present study, it may be further revealed that although the patent drug gliclazide was found to be highly

effective, as blood glucose lowering agent, but the efficacy of three Different combined form of herbal preparations was also seemed to be encouraging.

Gupta *et al.*, 2005 showed that in the present study, using infectious bursal disease virus (IBDV) infection in broiler chickens as a model, the antimicrobial and immune modulatory effects of the two preparations viz., dried leaves powder and essential oil of the *Ocimum sanctum* (Shyama Tulsi) medicinal plant were evaluated. Birds were treated with predetermined safe dose of dried leaves powder @ 200 mg/bird daily and essential oil @10µl/bird daily orally over a period of 25 days and infected experimentally at day 5 with 1 X 10^5 $TCID_{50}$ dose of Georgia strain of IBDV. A reduction in viral replication in the target organ viz. bursa of Fabricus was observed in the birds treated with dried leaves powder as evidenced by marked reduction in gross and microscopic lesions of IBD and the reduced virus titre in bursa. In these birds, immune potentialion as measured by increase in skin thickness for contact hypersensitivity response was also observed. However, the birds treated with dried leaves powder showed markedly lower antibody responses as measured by serum neutralization test and ELISA. This may be due to accelerated virus clearance in these chickens mediated through generalized enhanced cell mediated immune response. On the other hand, the birds treated with essential oil showed some toxic effects as evidenced by hemorrhages on thigh muscles. Thus the dried leaves powder of *O. sanctum* has the potentials to be effectively utilized as a feed supplement against some of the important poultry pathogens particularly IBDV, since it was found to inhibit the virus replication *in vivo*. However, the field applications of *Ocimum sanctum* will depend upon the results of studies in a larger population of birds and the outcome of similar studies against a number of other important pathogens of poultry.

Saxena *et al.*, 2004 conducted that type 2 diabetes has become a global epidemic. Modern medicines, despite offering a variety of effective treatment options, can have several adverse effects. Ayurveda, a science that uses herbal medicines extensively, originated in India. Of considerable interest is the adoption of

Ayurveda by the mainstream medical system in some European countries (e.g., Hungary), emphasizing this modality is increasing worldwide recognition. From ancient times, some of these herbal preparations have been used in the treatment of diabetes. This paper reviews the accumulated literature for 10 Indian herbs that have antidiabetic activity and that have been scientifically tested. Few of these herbs, such as Momordica charantia, Pterocarpus marsupium, and Trigonella foenum greacum, have been reported to be beneficial for treating type 2diabetes. Mechanisms such as the stimulating or regenerating effect on beta cells or extrapancreatic effects are proposed for the hypoglycemic action of these herbs.

Halim *et al.*, 2003 studied that combination (1:1) of water extract of dried powder of root and leaves (200 mg/kg body wt) of A. augusta and A. indica respectively was administered orally to alloxan diabetic rats once a day for 8 weeks. This treatment caused significant lowering of blood sugar in fasted as estimated by glucose tolerance test. The treatment resulted in a significant reduction in serumlipids. Aqueous extract also decreased the formation of lipid peroxides estimated as thiobarbituric acid reactive substance, (TBARS), and increased antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase) in erythrocytes. There was reduction in LPO as TBARS in heart, liver, kidney, and muscles. It also prevented decrease in body weight. Present study showed that Abroma augusta roots and A. indica leaves when given together as water extract had hypoglycaemic action and had better effect than given alone.

Sridhar *et al.*, 2003 studied that Effect of Neem leaf extract (NLE) administration was studied on clinical signs and haematological changes in broiler chickens. Neem leaf extract was prepared following boiling of 100 g leaves in one litre of drinking water for treated chicks. In NLE treated birds, mild depression, isolation of some birds and lesser body weight gain were observed during the study after 4 weeks and onwards. Haematological observations revealed lower values of haemogram (Hb, PCV and TEC) in NLE treated chicks 4 to 6 weeks post

treatment. However, there were no significant changes in TLC and DLC in treated birds.

Sundararaju *et al.*, 2003 studied that Dry and fresh leaves of ten locally available botanicals were tested against the root-lesion nematode, *Pratylenchus coffeae* in banana CVS Nendran and Rasthali under field conditions. Al1 the botanicals were effective in reducing the nematode population and subsequently increased the plant growth characters and yield compared to untreated control. Among the different botanicals tried, application of *Azadirachta indica*, *Datura stramonium, Crotolarza juncea* and *Vitex negundo* were found to be superior and effective in reducing the nematode population and increasing the yield significantly.

Rai *et al.*, 1997 showed that Tulsi leaf powder was fed at the 1% level in normal and diabetic rats for a period of one month to explore the effect on fasting blood sugar, uronic acid, total amino acids, and the lipid profile in serum and tissue lipids. The results indicated a significant reduction in fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids. In liver, total cholesterol, triglyceride and total lipids were significantly lowered. Total lipids were significantly reduced in kidney. In heart, a significant fall in total cholesterol and phospholipids was observed. All these observations indicate the hypoglycemic and hypolipidemic effect of Tulsi in diabetic rats.

CHAPTER 3
MATERIALS AND METHODS

3.1: Experimental design:

All the 40 chicken randomly divided into 4 groups (A, B, C and D) for assessing the efficacy of grinded plants leaves as growth promoter on broilers.

Chickens of group ‘A’: was kept as control and was not treated.

Chickens of group 'B': was treated with grinded Neem leaves @ 2gm in 1 liter water for consecutive six weeks.

Chickens of group 'C': was treated with grinded Nishyinda leaves @ 2gm in 1 liter water for consecutive six weeks.

Chickens of group 'D': was treated with 1gm grinded Neem leaves plus 1gm grinded Nishyinda leaves in 1 liter water for consecutive six weeks.

All the broiler of treated and control groups were closely observed for 42 days and following parameter were studied:

LAYOUT OF THE EXPERIMENT

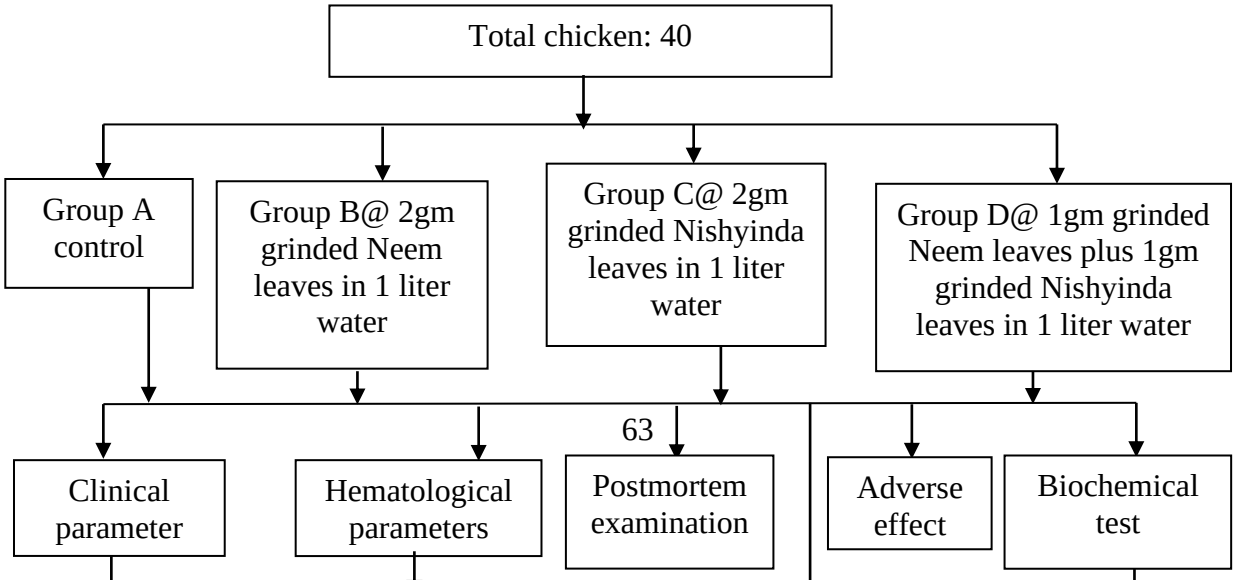

Fig: Layout of the experimental design (each group consisting of ten birds)

3.2: Collection of plant leaves:

Neem and Nishyinda leaves were selected for effectiveness as growth promoter on broiler. Mature and disease free Neem and Nishyinda leaves were collected from tree.

Fig: Nishyinda leaves(*Vitex negundo)* Fig: Neem leaves (*Azadirachta indica)*

3.3: Preparation of grinded leaves for supplementation:

Mature and disease free leaves were collected. After collection, leaves were washed in water and cut into small pieces. Then fine grind was made by using pestle and morter, 2 gm grinded leaves ware mixed with 1 liter of water and stored in refrigerator for further use.

Fig: Grinded Neem leaves

3.4: Collection and management of broiler chicks:

Broiler chicks collected from local market. The finally selected 40 chickens were allowed to acclimatize for 7 days in the experimental shed. The body weights of assigned chickens were taken with digital weight balance and the results were recorded. During acclimatization recommended feed and water supplied to the chicken.

Fig: Broiler chicks in experimental shed

3.5: Clinical examination:

(i) The effect of the Neem, Nishyinda and Neem plus Nishyinda grinded leaves on body weight, feed consumption was recorded.

(ii) Chickens under trial and control groups were weighed in weighing balance.

Fig: Broiler Chicks in treatment group (B)

Fig: Broiler Chicks in treatment group (C)

Fig: Broiler in growing stage in treatment group (D)

Fig: Broiler in growing stage in treatment group (A)

3.6: Hematological test:

Blood samples were collected from neck vein of broiler of both control and treated groups in day 21 and day 42 to study the effect of the Neem, Nishyinda and Neem plus Nishyinda grinded leaves and the following parameters were observed:

(a) Total erythrocyte count (TEC)

(b) Hemoglobin estimation (Hb)

(c) Packed cell volume (PCV)

(d) Erythrocyte sedimentation rate (ESR)

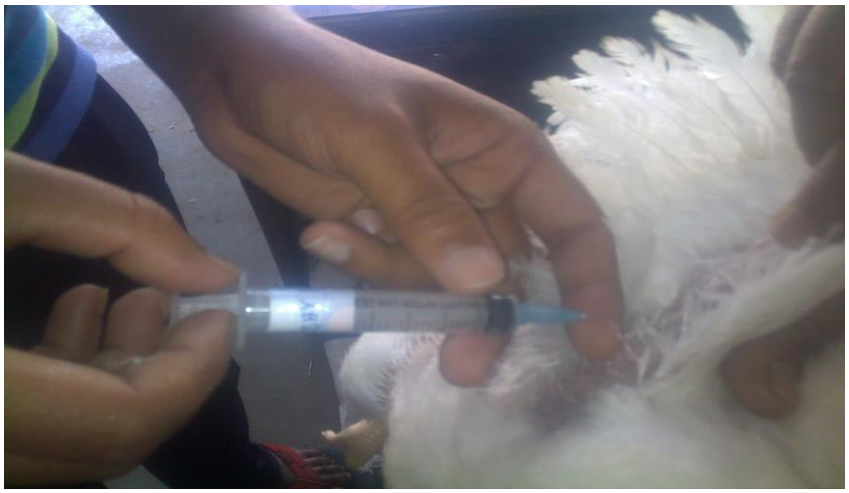

Fig: Blood collection from the wing vein (B)

Determination of Total erythrocyte count (TEC):

Total erythrocyte count was done following the method described by Lamberg and Rothstein (1977). Well-mixed blood sample was drawn with red blood cell diluting pipette exactly up to 0.5 marks of the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The free end of the pipette was wrapped around with the rubber tube stretching to both the ends and held with thumb and middle finger. The content of the pipette was mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. Then the counting chamber was placed with special cover glass under microscope using low power (10X) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle on the chamber under the cover glass. Taking 5 larger squares (4 in the 4 corners and the central one) of the central large square, the cells were counted from all the 80 small squares (16 x 5) under high power objectives (45X). After completion of counting, the total number of RBC was calculated as number of cells counted x 10, 000 and the result was expressed in million/µl of blood.

Determination of Hemoglobin concentrations (Hb):

The N/10 hydrochloric acid was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematinic mixture in the tube by hemolysing red blood cells by the action of hydrochloric acid (HCL). The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was

mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in gm %. The above procedure was matched by the Hellige hemometer method as described by Lamberg and Rothstein (1977).

Determination of Packed cell volume (PCV):

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then, the hematocrit or PCV was recorded by reading the graduation mark; the percent volume occupied by the hematocrit was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV\%} = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \text{ X } 100$$

Determination of Erythrocyte sedimentation rate (ESR):

The fresh anticoagulant blood was taken into the Wintrobe hematocrit tube by using special loading pipette exactly up to 0 marks. Excess blood above the mark was wiped away by sterile cotton. The filled tube was placed vertically undisturbed on the wooden rack for one hour. After one hour the ESR was recorded from the top of the pipette. The result was expressed in mm in 1st hour.

3.7: Biochemical test:

- Serum Glutamic Pyruvic Transaminase (SGPT) or Alanine Amino Transferase (ALT)
- Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspertate Amino Transferase (AST)

Determination of Serum Glutamic Pyruvic Transaminase (SGPT):

Before performing the test switch on "Reflotron" instrument when READY appears in the display, take a reagent carriers strip out of the vial. Close the vial immediately with the desiccant stopper. Remove the foil protecting the test area.

Take care not to over band the strip. By using "Reflotron", pipette, draw up the sample material (0.3 ml) avoiding the formation of bubbles and apply this as a drop to the center of the Red application zone without allowing the pipette to touch the zone.

Within 15 seconds open the flap, place the strip on the guide and insert the strip horizontally into the instrument until hearing a clock.

Close the flap, the display SGPT, confirms that the test specific magnetic code has been correctly read into the instrument.

The time before the result appears in displayed in seconds. After particular time the SGPT concentration displayed in IU/L for 37°C depending up on the reference temperature selected.

Determination of Serum Glutamic Oxaloacetic Transaminase (SGOT):

Before performing the test switch on "Reflotron" instrument when READY appears in the display, take a reagent carriers strip out of the vial. Close the vial immediately with the desiccant stopper. Remove the foil protecting the test area.

Take care not to over band the strip. By using "Reflotron", pipette, draw up the sample material (0.3 ml) avoiding the formation of bubbles and apply this as a drop to the center of the Red application zone without allowing the pipette to touch the zone.

Within 15 seconds open the flap, place the strip on the guide and insert the strip horizontally into the instrument until hearing a clock.

Close the flap, the display SGOT, confirms that the test specific magnetic code has been correctly read into the instrument.

The time before the result appears in displayed in seconds. After particular time the SGPT concentration displayed in IU/L for 37°C depending up on the reference temperature selected.

3.8: Postmortem examination for side effect:

Two broilers from every group were slaughtered to see if there were any pathological changes present in day 21 and day 42 of treatment. There was no significant pathological change in any internal organs of the broiler of treated groups.

Fig: Postmortem examination of broiler

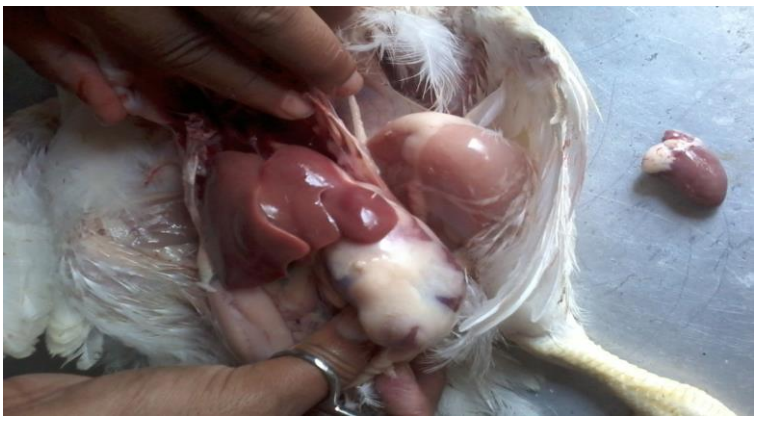

Fig: Examination of organ of broiler

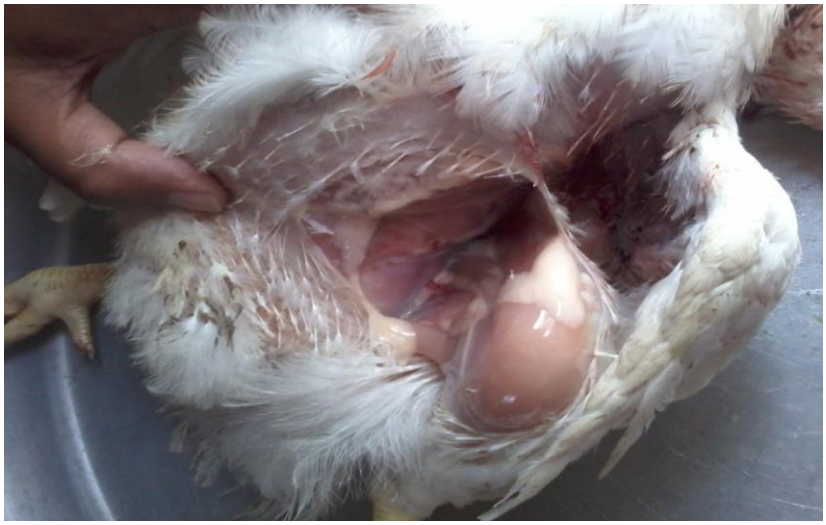

Fig: Examination of organ of broiler

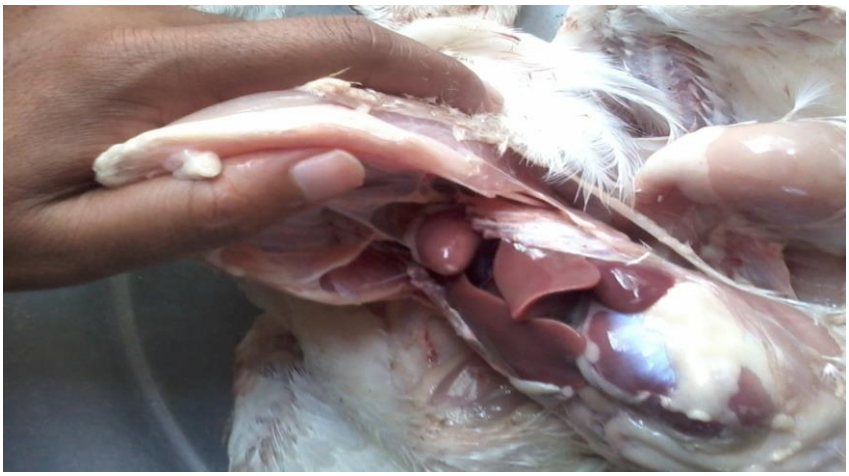

Fig: Examination of organ of broiler

3.9: Fecal examination:

For determinations of infectivity, fecal samples were collected from every group in 20, 30 and 42 day and parasitic eggs were counted by direct smear method.

Direct smear method:

Material: Glass slides, cover slip, tooth-pick, detergents and a compound microscope.

Method: A small quantity of feces put on slides with the help of tooth-picks, mixed with a drop of water, spread out, clean the mixture by removing the coarse particle of the feces, covered with a slip and examined directly under microscope with low power. At least three slides from each fecal sample were examined.
At least 5 gm of feces were collected.

3.10: Statistical analysis:

The data were analyzed statistically between control and treated groups of broiler by the analysis of variance (ANOVA) technique in completely randomized design.

CHAPTER 4
RESULTS AND DISCUSSION

This experiment was conducted to study the efficacy of grinded Neem (*Azadirachta indica*) and grinded Nishyinda (*Vitex nigundo*) leaves as a growth promoter in broiler and was held under the Department of Physiology and Pharmacology. 40 day old chicks were randomly divided into 4 groups (A, B, C and D) for assessing the efficacy of grinded plants leaves as growth promoter on broilers. The experimental units were kept on a floor litter system in separate pens. Equal amount of ration was offered to the birds twice a day and left over feed was collected to calculate feed consumption of the birds. Fresh and clean water was available at all the time. The experiment was conducted according to the completely randomized design and data about final body weight, weight gain, feed consumption and mortality were recorded during the experimental period (1-6 weeks of age).

Physical appearance:

The physical appearance of birds of all the treated groups (with Neem, with Nishyinda and with Neem plus Nishyinda) was better than the control group. The

birds of the treated groups shown good response to attendance, better glossy plumage and they took feed more rapidly than the control groups.

Body weight:

Body weights of different groups of birds are presented in Table - 1. Broiler treated with Neem, Nishyinda and Neem plus Nishyinda supplementation showed an increased body weight gain than control group (without treatment).

Table1. Initial and final live weight, weight gain, feed consumption and feed conversion ratio of broilers fed at different levels of grinded Neem and grinded Nishyinda leaves from 1 to 6 weeks of age:

Groups	Initial live weight (gm) on day 7	Final live weight (gm) on day 42	Weight gain (gm)	Feed consumption (gm)	Feed conversion ratio (gm feed consumed/gm weight gain)
A	167.00a ± 2.00	1610.00d ± 10.00	1443.00d ± 8.00	3000.00d ± 0.00	2.0750a ± 0.015
B	166.00a ± 4.00	1830.00b ± 10.00	1664.00b ± 6.00	3250.00b ± 0.00	1.985bc ± 0.025
C	169.00a ± 2.00	1720.00c ± 15.00	1551.00c ± 13.00	3100.00c ± 0.00	1.995b ± 0.015
D	168.00a ± 0.00	1910.00a ± 5.00	1742.00a ± 5.00	3350.00a ± 0.00	1.915c ± 0.005

Note: Values followed by same superscripts in the same column are not statistically significant ($p>0.05$), different superscripts indicate that difference is significant ($P<0.05$). In this and other tables, A = control (without treatment), B= 2gm grinded Neem leaves, C = 2gm grinded Nishyinda leaves, D= 1gm grinded Neemplus1gm grinded Nishyinda leaves.

Table 1.revealed that-

In Group A initial live wt. 167 gm, final live wt. 1610 gm, weight gain 1443 gm and Feed conversion ratio (FCR) 2.07

In Group B initial live wt. 166 gm, final live wt. 1830 gm, weight gain 1664 gm and FCR 1.95

In Group C initial live wt. 169 gm, final live wt. 1720 gm, weight gain 1551 gm and FCR 1.99

In Group D initial live wt. 168 gm, final live wt. 1910 gm, weight gain 1742 gm and FCR 1.92

The birds of group D using ration supplemented with grinded 1gm Neem plus grinded 1gm Nishyinda leaves utilized their feed statistically significantly ($P<0.05$) more efficiently among the other groups.

The birds using ration supplemented with (1gm Neem plus1gm Nishyinda) (Group D) leaves gained the highest live weight among the other groups and it is significant at 5%($p<0.05$) level. Supplementation of grinded Neem plus Nishyinda (D) leaves in the treatment caused improvement in the feed efficiency as compared to that of other group. Similarly, Nemade *et al.*, 1993 reported increase in feed efficiency in Neem and Nishyinda fed groups, which is in agreement with the findings of the present study.

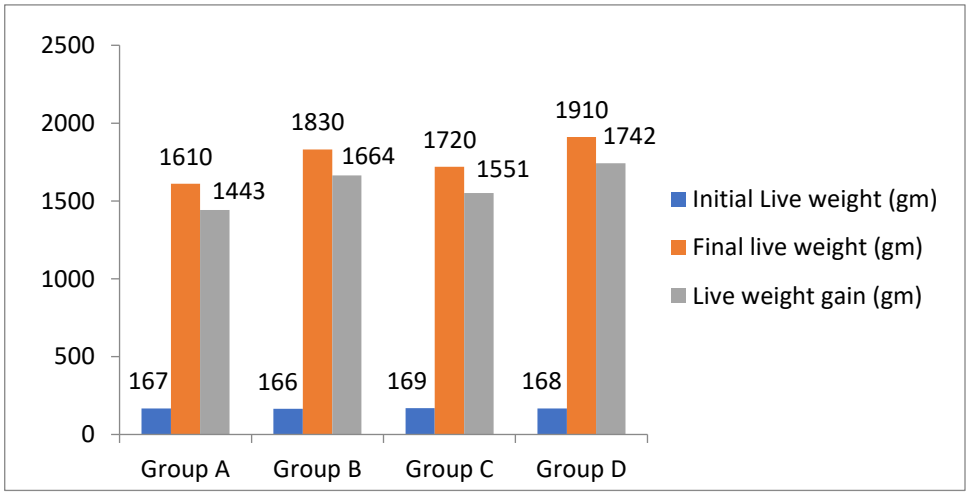

Fig. Weight gain in different groups

Dressing percentage and relative giblet weight:

Dressing percentage and relative giblet weight of different groups of birds are presented in (Table 2). There was no significant different of all the groups.

Table 2. Dressing percentages, relative giblet weight (heart, gizzard, liver and spleen) and pancreas weight of broilers fed at different levels of grinded Neem and grinded Nishyinda leaves from 1-6 weeks of age:

Groups	Dressing percentage	Relative heart weight (gm)	Relative gizzard weight(gm)	Relative liver weight (gm)	Relative spleen weight (gm)	Relative pancreas weight (gm)
A	63.57b ± 1.010	0.47b ± 0.040	1.42c ± 0.100	2.57c ± 0.045	0.13b ± 0.005	0.26c ± 0.025

B	65.68ab ± 0.505	0.57b ± 0.050	1.68b ± 0.020	2.78b ± 0.045	0.17ab ± 0.010	0.34ab ± 0.005
C	64.34b ± 1.005	0.56b ± 0.015	1.61b ± 0.030	2.73b ± 0.025	0.15ab ± 0.005	0.30bc ± 0.005
D	67.14a ± 0.285	0.71a ± 0.025	1.97a ± 0.010	2.91a ± 0.030	0.20a ± 0.010	0.38a ± 0.015

Note: Values followed by same superscripts in the same column are not statistically significant (p>0.05), different superscripts indicate that difference is significant (P<0.05).

- Relative weight (gm) = Weight of organ/ Live body weight of bird X 100
- Dressing %= Dress weight of bird/Live weight of bird X 100

Table 2. revealed that-

Statistical analysis of the data did not show any difference (P<0.5) between the dressing percentages of the birds of different feeding groups.

Statistical analysis of the data did not show any difference between the relative gizzard weights of the birds of different feeding groups.

Statistical analysis of the data did not show any difference between the relative spleen weight of the birds of different feeding groups using ration with or without supplementation of Neem and Nishyinda leaves.

Economics of Production:

Cost of different groups of bird is presented in (Table 3).

Table 3. Data showing economics of broiler production kept under different treatment groups from day old chick to 6 weeks of age:

Description	A	B	C	D
Cost/chick (Taka)	46.00	46.00	46.00	46.00
Average feed consumed (Kg)/chicks/42 days	3.000	3.250	3.100	3.350
Feed price/kg (Taka)	44.50	44.50	44.50	44.50
Cost of herbal growth promoters (Taka)	0.00	2.00	2.00	2.00
Total feed cost (Taka)/Broiler	133.50	146.62	139.95	151.07
Miscellaneous cost (Taka)	10.00	10.00	10.00	10.00
Total cost/Broiler (Taka.)	189.50	202.62	195.95	207.07
Average live weight (Kg)/Broiler	1.610	1.830	1.720	1.910
Sale price/Kg live wt. (Taka.)	135.00	135.00	135.00	135.00
Sale price/broiler (Taka)	217.35	247.05	232.20	257.85
Net profit/broiler (Taka.)	27.85	44.42	36.25	50.78
Profit/ Kg live weight (Taka)	17.29	24.27	21.07	26.58

Table 3. revealed that-

The average rearing costs of broiler are kept under different treatment groups viz. A, B, C,D was 189.50Tk, 202.62Tk,195.95Tk and 207.07Tk respectively. Miscellaneous cost summed up Tk10 per broiler, which included the estimated cost of electricity cost of labor, litter, disinfectant. The average live weight/broiler in group A,B,C and D was 1.610 kg, 1.830 kg, 1.720 kg and 1.910 kg respectively. The broiler was sold in live weight basis at the rate of Tk 135/kg. The net profit/Kg live weight in the respective group was found to be taka 17.29, 24.27, 21.07 and 26.58 respectively. The level of Neem and Nishyinda grinded leaves used in the ration exhibited their effect on the profit margin of the broiler.

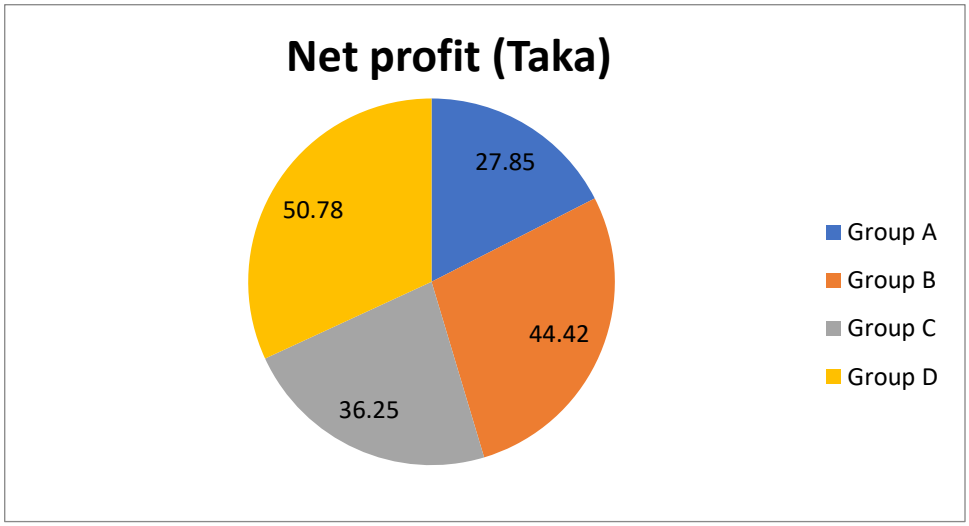

Fig. Net profit per broiler (Tk)

Hematological and Biochemical parameter:

Table 4. Study of grinded Neem and Nishyinda leaves on hematological and biochemical parameter of broiler on day 21

Groups	TEC (million/mm^3) (Mean ± SEM)	Hb (gm/dl) (Mean ± SEM)	PCV (%) (Mean ± SEM)	ESR (mm/1st hour) (Mean ± SEM)	SGPT (IU/L) (Mean ± SEM)	SGOT (IU/L) (Mean ± SEM)
A	189.30b ± 0.600	5.925c ± 0.08	15.38c ± 0.125	10.86a ± 0.195	24.55a ± 0.350	28.13a ± 0.175
B	194.50a ± 0.400	6.39a ± 0.04	17.95a ± 0.350	8.69c ± 0.110	24.00ab ± 0.100	26.32bc ± 0.575
C	190.79b ± 0.59	6.15b ± 0.10	16.69b ± 0.515	9.40b ± 0.200	23.32b ± 0.375	27.22ab ± 0.125

D	195.995a ± 0.33	6.42a ± 0.04	18.89a ± 0.110	8.48c ± 0.125	22.18c ± 0.320	25.95c ± 0.150

Note: Values followed by same superscripts in the same column are not statistically significant (p>0.05), different superscripts indicate that difference is significant (P<0.05).

Table 5. Study of grinded Neem and Nishyinda leaves on hematological and biochemical parameter of broiler on day 42

Groups	TEC (million/mm^3) (Mean ± SEM)	Hb (gm/dl) (Mean ± SEM)	PCV (%) (Mean ± SEM)	ESR (mm/1st hour) (Mean ± SEM)	SGPT (IU/L) (Mean ± SEM)	SGOT (IU/L) (Mean ± SEM)
A	237.48d ± 1.125	6.80d ± 0.050	15.98d ± 0.145	11.81a ± 0.245	27.03a ± 0.205	30.44a ± 0.335
B	291.02b ± 0.885	8.04b ± 0.115	21.46b ± 0.155	7.64c ± 0.160	22.54c ± 0.365	24.39c ± 0.265
C	285.01c ± 0.110	7.13c ± 0.020	19.19c ± 0.015	9.40b ± 0.200	23.88b ± 0.075	26.28b ± 0.175
D	295.74a ± 0.585	8.74a ± 0.085	22.930a ± 0.745	6.38d ± 0.245	21.43d ± 0.025	25.11c ± 0.005

Note: Values followed by same superscripts in the same column are not statistically significant (p>0.05), different superscripts indicate that difference is significant (P<0.05).

Hematological parameter:

A. Total Erythrocyte Count (million/ mm^3):

Total erythrocyte count is presented in(Table 4& 5)in day 21 and in day 42. The values of TEC in all treated groups and control group were more or less similar and the values were within the normal range. The highest TEC was recorded in Group D and lowest in Group A. Although these values show a little fluctuation they were not statistically significant (p>0.05).

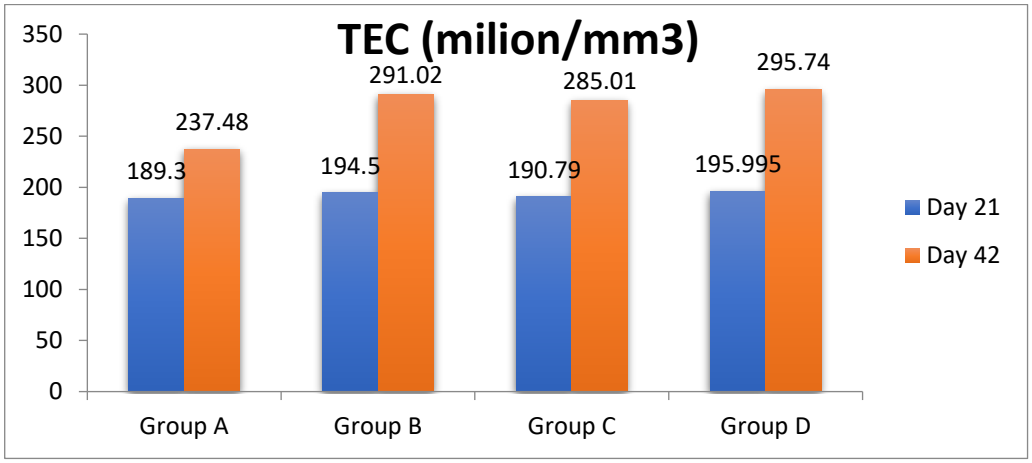

Fig.TEC levels in different group

B. Estimation of Hemoglobin (gm/dl):

Hemoglobin content is presented in (Table 4 & 5) in day 21 and in day 42. The values of Hb in all treated groups and control group were more or less similar and the values were within the normal range. The highest Hb was recorded in Group D and lowest in Group A. All the data were statistically significant ($p<0.05$).

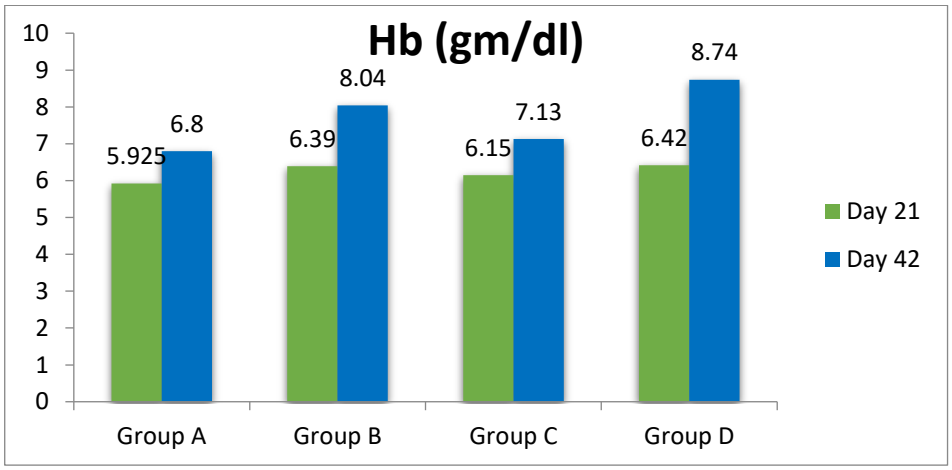

Fig. Hb levels in different group

C. Packed Cell Volume (%):

Packed cell volume is presented in (Table 4 & 5) in day 21 and in day 42. The values of PCV in all treated groups and control group were more or less similar and the values were within the normal range. The highest PCV was recorded in Group D and lowest in Group A. Although these values show a little fluctuation they were statistically significant ($p<0.05$).

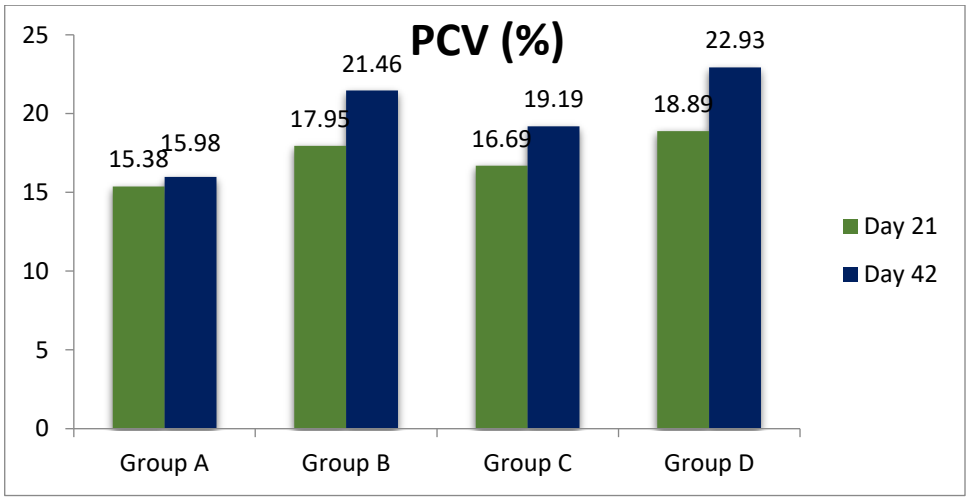

Fig. PCV levels in different group

D. Erythrocyte Sedimentation Rate (mm/1st hour):

Erythrocyte sedimentation rate content is presented in (Table 4 & 5) in day 21 and in day 42. The values of ESR in all treated groups and control group were more or less similar and the values were within the normal range. The highest ESR was recorded in Group A and lowest in Group D. Although these values show a little fluctuation they were not statistically significant (p>0.05).

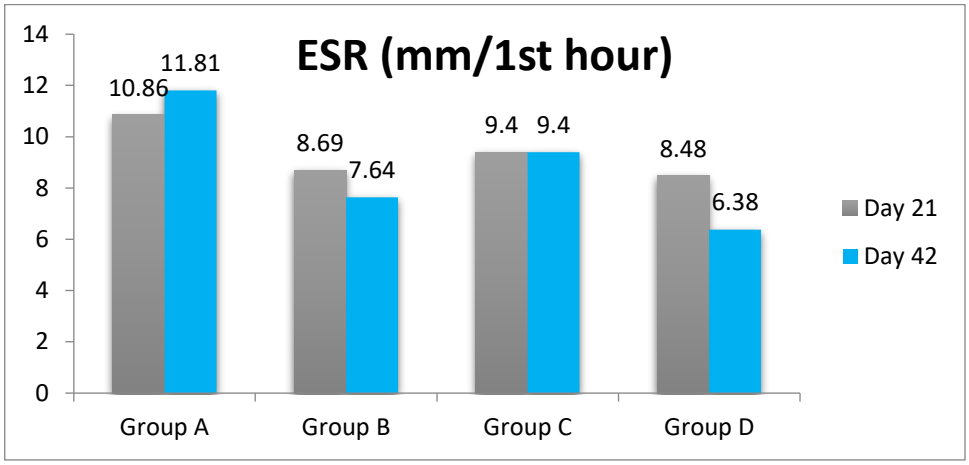

Fig. ESR levels in different group

This study has revealed that there is an inverse relationship between grinded Neem plus Nishyinda (D) leaves doses and on the body weight (Table1) and also on the hematological effects in broiler (Table 4 & 5). This agrees with Nagalakshmi *et al.*, (1996) and Gowda *et al.*, (1998) that Neem plus Nishyinda bitters possess a strong influence on hematological traits particularly PCV and Hb of subjects, depending on their nutritional status. The performance of birds fed Neem plus Nishyinda (D) showed significantly better performance as compared to the other group. These results coincide with those of Chakravarty *et al.*, (1991) who achieved highest body weight gain and best feed conversion ratio as compared to control when offered Neem plus Nishyinda (D) leaves extract to broilers from 1 to 6 weeks.

Biochemical Parameter:

A. Serum Glutamic Pyruvic Transaminase (IU/L):

SGPT level is presents in (Table 4& 5) in days 21 and days 42. The values of SGPT in all treated groups and control group were more or less similar and the

values were within the normal range. The highest SGPT was recorded in Group A and lowest in Group D. Although these values show a little fluctuation they were statistically significant ($p<0.05$).

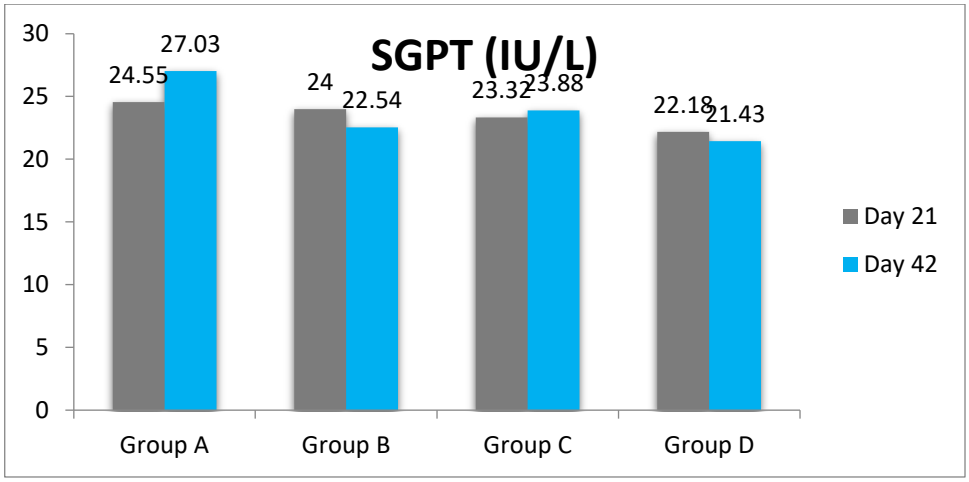

Fig. SGPT levels in different group

B. Serum Glutamic Oxaloacetic Transaminase (IU/L):

SGOT level is presented in (Table 4) in days 21 and days 42. The values of SGOT in all treated groups and control group were more or less similar and the values were within the normal range. The highest SGOT was recorded in Group A and lowest in Group D. Although these values show a little fluctuation they were statistically significant ($p<0.05$).

The greatly reduced SGPT and SGOT titer level as observed in the present study implies good health condition with less damage of muscle cell.

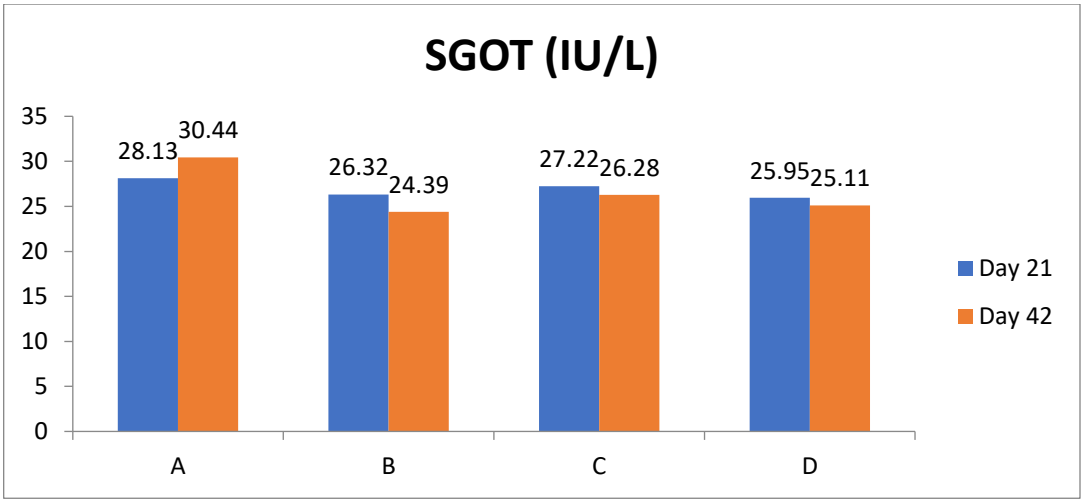

Fig. SGOT level in different group

GUT biotic Environment:

Feces examination result is given in (Table 6).

Table 6. Study of grinded Neem and Nishyinda leaves on GUT biosis of broiler (feces examination)

Groups	No. of eggs (Mean ± SEM) In day 20	No. of eggs (Mean ± SEM) In day 30	No. of eggs (Mean ± SEM) In day 42
A	6.50b ± 0.50	7.50a ± 0.50	8.50a ± 0.50
B	7.50ab ± 0.50	5.50b ± 0.50	2.00b ± 0.00
C	7.50ab ± 0.50	4.50b ± 0.50	1.50b ± 0.50
D	8.50a ± 0.50	2.50c ± 0.50	0.00c ± 0.00

Note: Values followed by same superscripts in the same column are not statistically significant ($p>0.05$), different superscripts indicate that difference is significant ($P<0.05$).

Table 6. revealed that–

Parasitic eggs were in Group D>Group B> Group C> Group A in day 20, in day 30 it was Group A> Group B>Group C> Group D and in day 42it was Group A> Group B>Group C> Group D. It shows that in treatment groups it gradually decreases and in control group it gradually increases. It means that this grinded leaves has anti-parasitic activity on broiler which ensure lower mortality rate and higher body weight gain. Treatment with grinded Neem plus Nishyinda (Group D) leaves gives the better result than grinded Neem (Group A) and grinded Nishyinda (Group B) leaves group. These results may be due to antimicrobial and anti-

protozoal properties (Kale *et al*., 2003) of Neem and Nishyinda leaves, which help to reduce the microbial load of birds, improved the feed consumption and feed efficiency of the birds.

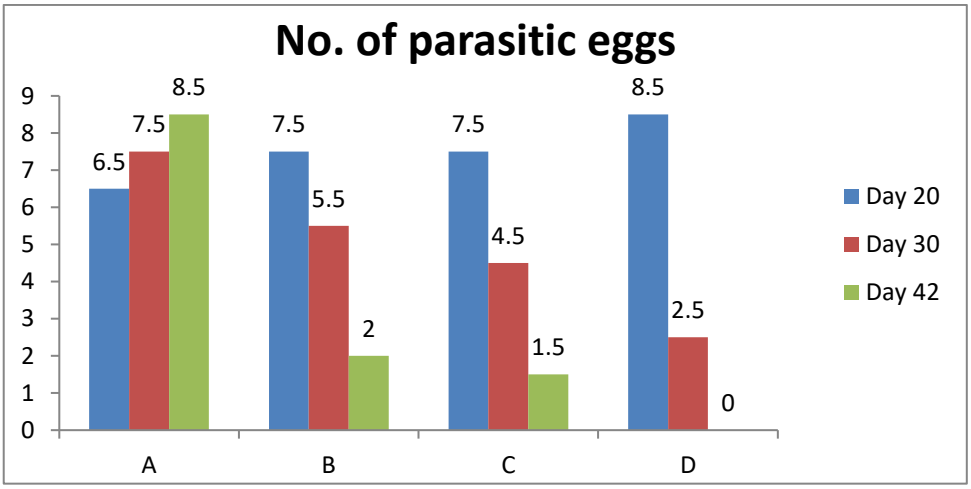

Fig. Parasitic eggs in different group

Neem plus Nishyinda (D) grinded leaves suppresses pathogenic bacteria including *Staphyloccoccus aureus, Salmonella paratyphi* and *Klebsiellas pneumoniae*. In control group (Group A), 3 birds were died due to management problem viz. lack of proper ventilation, humidity etc and parasitic infestation. On the other hand, all the birds of the treatment groups (Group B, C and D) were alive.

CHAPTER 5
SUMMARY AND CONCLUSION

Forty healthy commercial broilers were equally divided into four groups (n=10) to carry out this research work. In this experiment, grinded Neem and Nishyinda leaves were studied in terms of growth promoter on broilers because we showed that these herbal plants are available, cost effective and produced broilers are free from any drug residual effect and suitable for human *consumption*. In this

research work, the continuous treatment with grinded Neem (*Azadirachta indica*) and grinded Nishyinda (*Vitex negundo)* leaves produced a significant ($p<0.05$) increased of the live body weight. It is concluded that supplementation of 1gm grinded Neem plus 1gm grinded Nishyinda (D) leaves per liter water of treatment groups caused significant increase in live body weight and feed efficiency as compared to that of other groups of broilers. From this experiment we found that, between the control group and the treatment group of birds, the Neem plus Nishyinda (D) groups are more profitable than any other groups. Hemoglobin content significantly ($p<0.01$) increased and ESR content significantly ($p<0.05$) decreased with Neem plus Nishyinda (D) supplementation. No significant ($p>0.05$) differences were observed among the treatment groups in case of PCV and TEC values in respect to the control group after treatment. The SGOT level was decreased significantly ($p<0.05$) with grinded Neem plus grinded Nishyinda (D) leaves and SGPT level was also decreased with the same kind of supplementation, but both SGOT and SGPT level increased in case of control (A) group. We also found that, in the treatment groups there is significant decrease of parasitic eggs, but in case of control group there is increase of parasitic eggs. There was no significant pathological change in any internal organs of the broiler of treated groups. From the present field and laboratory trial, it can be concluded that combine supplementation of 1gm Neem plus 1gm Nishyinda (D) per liter water is highly beneficial for broiler growth without making any potential hazards of broiler and our formulations could be used as an alternative to growth promoters. Further studies are necessary to see any adverse effect in relation to histopathology before making a definite conclusion.