COMPARATIVE EFFICACY OF PINEAPPLE (*ANANUS COMOSUS*) STEM AND IVERMECTIN AGAINST GASTRO-INTESTINAL NEMATODIASIS IN CATTLE IN DINAJPUR DISTRICT

A Thesis

by

DR. MD. RASHED KAMAL
Registration No. 1105125
Semester: July-December/ 2012

MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
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Submitted to the
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Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, in partial fulfillment of the degree of

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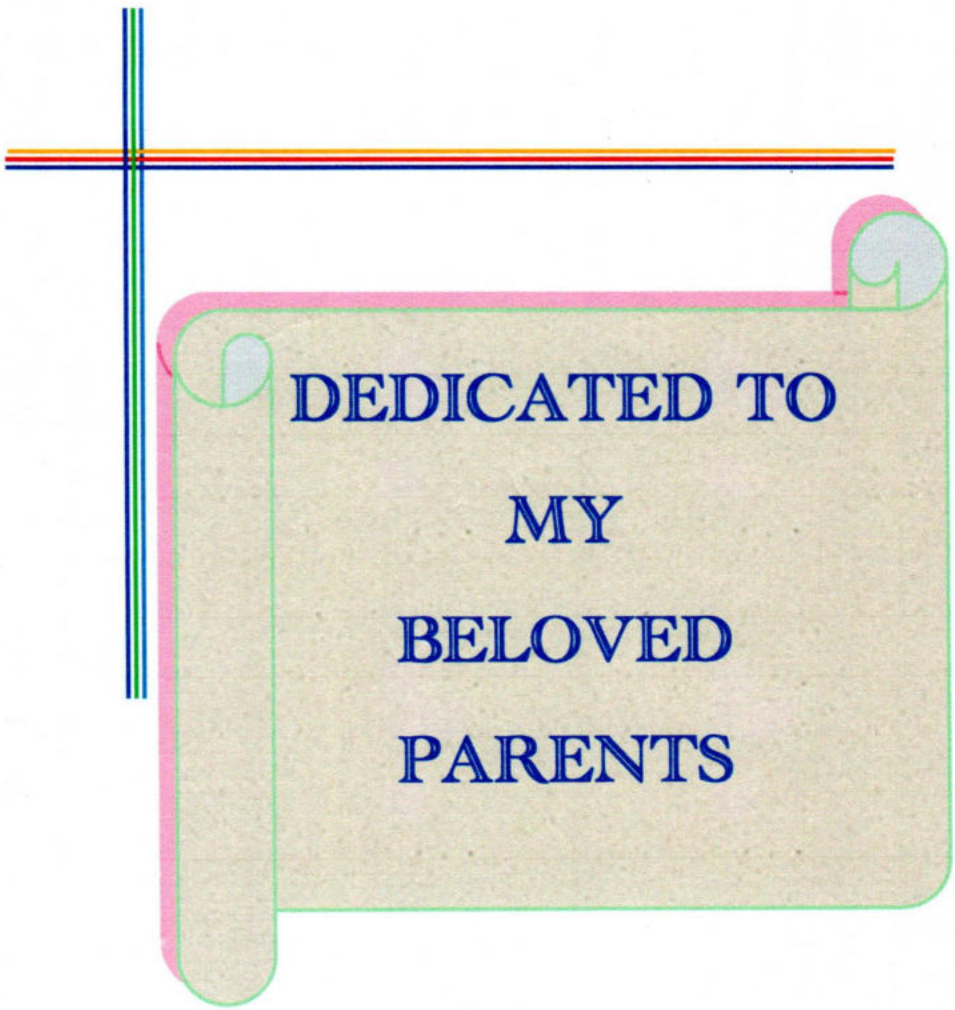
DECEMBER, 2012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PARENTS

ACKNOWLEDGEMENTS

All praises are to Almighty Allah, Who enabled the author to complete the research work for the degree of Masters of Science (M.S.) in Veterinary Pharmacology and to prepare this manuscript.

The author would like to take the opportunity to express his immense indebtedness and deepest sense of gratitude to his venerable supervisor Dr. Fahima Binthe Aziz Assistant Professor, Department of Pharmacology, Faculty of Veterinary and Animal science (FVAS), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur innovative suggestions, constant supervision, timely instructions and inspirations throughout the tenure of the research work. His insight and professional skill have made distinct contribution to complete this piece of research work and to prepare this thesis.

The author expresses his high appreciation to his co-supervisor Dr. Md. Nazrul Islam Assistant Professor, Department of Pathology and Parasitology, Faculty of Veterinary and Animal science (FVAS) , Hajee Mohammad Danesh Science and Technology University (HSTU) , Dinajpur for his valuable advice, constructive criticism, provision of laboratory facilities and factual comments in upgrading the quality of the manuscript.

The author also express his deepest sense of gratitude to his respect teacher, Dr. Md. Bazlar Rashid, Assistant Professor and Chairman, Department of Physiology and Pharmacology and Dr. Md. Mahmudul Hasan, Lecturer, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU) , Dinajpur for their encouragement and cordial co-operation through out the course of the study.

The author takes this opportunity in expressing his heartfelt thanks to Dr. Mst. Kamrunnaher Akter, Dr. Md. Anisur Rahman, Dr. Md. Ilius Ali, Dr. Md. Washim Akram for their help, constant inspiration encouragement.

The author expresses many thanks to all the official and laboratory staffs of the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, for their precious help during the research work.

The author feels much pleasure to convey the profound thanks to his friends and well wishers for their co-operation, cheerfulness and help during the on going of the research.

Finally, the author expresses his deepest sense of respect to his beloved Parents for their blessing, inspiration, advice and moral supports in all phases of his higher studies.

The Author

ABSTRACT

An experiment was carried out in the laboratory of the department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur and Asia Dairy Farm, Boroil, Gopalgonj,Dinajpur, during the period from July to December/2012. 15 cattle were randomly divided into 3 groups (A, B and C). Group A was kept for control, Group B was treated with Pineapple stem and Group C was treated with the Ivermectin. Those two were screened for assessment their comparative efficacy against gastro-intestinal nematodiasis in cattle in Dinajpur District. Pineapple stem was used at a dose of 150mg/kg bwt for seven consecutive days and Ivermectin at a dose of 200µg/kg bwt as a single dose. Efficacy was recorded as compared to control on the basis of fecal egg count. Ivermectin showed 100% efficacy within 28th days of treatment. On the other hand, Pineapple stem showed 80% efficacy on 28th day of treatment. Weekly observations were recorded for live body weight, weekly gain in weight, weekly feed consumption, feed efficiency and blood parameters of cattle for six months. All the treatment groups B (106.01) and C (1.25) recorded significantly higher means for live body weight than that of control A (90) group.

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ABBREVIATIONS

µg	Microgram
CHO	Carbohydrate
cm	Centimeter
CNS	Central nervous system
Cone	Concentration
Cr	Creatinine
Cu	Copper
Cumm	Cubic millimeter
d.w	Drinking water
ESR	Erythrocyte sedimentation rate
et al.	Et Alia (associates)
fe	Iron
Fig.	Figure
gm	Gram
GOT	Glutamate Pyruvate Transaminase
GPT	Hemoglobin
Hb	Hydrochloric acid
HCI	Mercury
Hg	Mercuric chloride
HLTH	Health
HSTU	Hajee Mohammad Danesh Science and Technology University
IP	Intraperitoneal
J.	Journal
Kg	Kilogram
lit	Litre
Ltd.	Limited
me	Mercuric chloride
mg	Milligram
ml	Milliliter
mm^3	Cubic millimeter
mmc	Methyl mercuric chloride
Mn	Manganese

Mo	Molybdenum
Mol	Mole
No.	Number
Pb	Lead
PBS	Phosphate Buffer saline
PCB	Polychlorinate biphenyl
PCV	Packed cell volume
ppm	Parts per million
rpm	Rotation per minute
Sc	Subcuteneous
SE	Standard error
Se	Selenium
UN	Urea nitrogen
Vol.	Volume
Zn	Zinc

CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

Gastro-intestinal (GI) nematodes remain a serious health problem in ruminants, in Bangladesh. Studies on animals reported prevalence of GI nematodes ranging from 65 – 100 %, and mortality due to parasitic gastro-enteritis ranging from 5 – 17 % (Kaneene *et al.,* 1985, Gorman *et al.*, 1986, Meister *et al.*, 1993).

Parasites can impose a great burden on the welfare of ruminants, affecting their growth and productivity. Being such a significant threat to animal health, it is important to provide comprehensive information on environmentally, friendly and economically viable options for treatment of these internal parasites.

Recently much interest in the field of medicinal plants has been grown throughout the world. Many countries have already come to realize the medicinal plants as a potential means of therapeutic agent and also their availability and cost effectiveness.

In developed countries the principles of controlling helminth parasites are based on pasture, barn management and protective treatments but in Bangladesh where animals are mainly maintained in mixed farming system with virtually no pasture land for grazing, these methods have limitation to control parasites. So, protective treatments with routine dosing of anthelmintics and dipping with ectopesticides would be the choice of controlling the parasites in animals of Bangladesh.

In Bangladesh many drugs are being used for long time to combat parasitic infection in livestock. A large number of anthelmintics are now available in the market. The indiscriminate use of anthelmintics made the parasites to be resistant against the drug, which have been reported by experts throughout the world including Bangladesh (Hannan, 1997). So, the scientist should have to back the traditional uses.

The main difficulty in parasite control is the contamination of enclosures with eggs and infective larvae, due to the permanent grazing and high stocking densities (Goossens *et al.*, 2005a). Therefore, parasite control relies mainly on the regular use of anthelmintics. Oral administration is preferred, because immobilization is not required. Compounds of the benzimidazoles and avermectin groups are most commonly used (VAN BOXSEL *et al.*, 1998). We found benzimidazoles to be effective against GI-nematodes in most herds of ruminants; however, indication of benzimidazole resistance development was demonstrated (GOOSSENS *et al.*, 2005b). Avermectin compounds such as ivermectin are used extensively since 1981 in domesticated ruminants. Their major advantages are the broad-spectrum activity against endo- and ectoparasites, A disadvantage is the development of ivermectin resistance, mainly in strongylids of small ruminants (GILL and LACEY, 1998) and to a lesser extent of cattle (FIEL *et al.*, 2001).

There are various types of ivermectin which are imported from abroad and very costly, having side effects and they are not equally active on all stages of life-cycle of ectoparasites.

Ivermectin was the safest and more effective for the treatment of endo and ectoparasites. The chemicals used against ectoparasites are hazardous for both man and animals. The poor farmers are illiterate. They can not apply insecticides on the animal body properly. In addition, there is no report on the toxic and residual effect of ivermectin.

The Agro-ecological and geo-climatic condition of Bangladesh favors the survival, multiplication, spread and perpetuation of animal parasites. Moreover animal management and managerial system play an important role in high degree of occurrence of parasitic disease in Bangladesh.

There are several indigenous medicinal plants (Ata, Neem, Pineapple, Tobacco, Sivdes, 1980; Nath, 1983; Mostofa, 1983; Safique, 1989; Hossain, 1994) have anthelmintics action and used against both ecto and enodparasites in Bangladesh

(Mostofa, 1983; Safique, 1983; Mannan, 1997). India (Dutta and Hazarika, 1976). Thailand and several counties so, if the uses indigenous medicinal plant increases as anthelmintics instead of imported drugs the country will be benefited and save her hardly earned foreign currency.

Objectives:

1. To study the comparative effects of Pineapple stem and Ivermectin against gastro-intestinal nematodiasis in cattle.
2. To study the effects of Pineapple stem and Ivermectin on blood parameters.
3. To serve a cost effective and easily applicable endoparasiticide to the farms as well as animals.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Avcioglu, *et al.* (2011) reported that a study was made to investigate efficacy of eprinomectin pour-on against to *Toxocara vitulorum* in cattle. In the study, 16 cattle naturally infected with *T. vitulorum* were divided into two groups as treatment (eight cattle) and control (eight cattle). Eprinomectin (0.5 mg/kg, Eprinex, Merial) was given to treatment group cattle, and eggs per gramme were determined in the faeces on the day of pre-treatment and the second, third, fourth, fifth, sixth, 14th and 28th days of post-treatment. No side effects associated with nervous, respiratory and gastrointestinal systems were observed. In conclusion, eprinomectin was determined to be 100% effective against *T. vitulorum*. This is the first study to evaluate the efficacy of eprinomectin against a natural *T. vitulorum* infection in cattle.

El-Gendy *et al.* (2010) showed that this experiment was carried out to determine the effect of adding 0, 0.25, 0.50, 0.75, and 1% of dry thyme *(Thymus vulgaris)* and sage *(Salvia officinalis)* leaves to the ration of Nile tilapia (*Oreochromis niloticus*) on growth performance, feed and nutrient utilization, whole body composition, hematogram, protein profile and the activity of serum enzymes. Fingerlings of 15g average weight were stocked in 27 glass aquaria (105 x 32 x 40cm) at a rate of 15 fish in each. Fish in each aquarium were handly fed on the experimental pelleted feeds twice daily, six days a week at a rate of 3% of body weight daily for a period of 105 days. The results showed that, nutrient utilization, growth performance, hematogram, protein profile and activity of serum enzymes were improved with adding the thyme at level of 0.25 and 0.5% and sage at 0.5% and 0.75%. Also, fish fed rations containing 0.25, 0.5, 0.75 and1% thyme or 0.75, and 1% sage had significantly ($P<0.05$) lower body fat, ash and energy contents but significantly ($P<0.05$) higher dry matter and body protein content. There were no significant mortalities among fish groups during the experimental period. Therefore, the levels of 0.25 or 0.5% thyme and 0.25, 0.5 or 0.75% sage are recommended as growth

promoters for Nile tilapia due to their favorable effects on growth performance and health status.

Maniruzzaman *et al.* (2010) studied that was conducted in 50 milk samples collected from apparently healthy lactating buffalo-cows during the period of July to October 2009 for isolation, identification of bacterial flora on basis of their morphology, staining, cultural and biochemical properties. Antibiotic sensitivity of the isolated organisms was also studied. Out of 50 milk samples examined for bacterial isolates. Total isolates were 40, out of which *Lactobacillus* spp. was 10 (25%), *Bacillus* spp. 10 (25%), *Staphylococcus* spp. 13 (32.5%), *E. coli* 5 (12.5%) and two 2 (5%) isolates were unclassified. Results of antibiotic sensitivity in this investigation showed that *Lactobacillus* spp. and *Bacillus* spp. were more or less sensitive to erythromycin, azithromycin, and nalidixic acid but resistant to ampicillin, penicillin, trimethoprim, amoxicillin and metronidazole. *Staphylococcus* spp. was more or less sensitive to erythromycin, azithromycin, ampicillin, amoxicillin, nalidixic acid and penicillin but resistant to metronidazole and trimethoprim. *E. coli* was more or less sensitive to all types of antibiotics used for experiment. Judging from the parameter it was concluded that bacteria of fresh milk are not harmful but pathogenic bacteria may invade milk through various sources, which is harmful.

Bag *et al.* (2010) studied that the present study was performed in the Department of General Animal Science and Animal Nutrition, CVASU during the period from April 2008 to January 2009 to characterize RCC in Chittagong district in Bangladesh. A total of 50 cattle were examined where 15 were male and 35 were female. It was observed that the coat color of these cattle was deep red to yellowish red to whitish red. The color of other parts of the body like horn, hoof, ears, eyeball, eyebrow, vulva and tail switch were also near to red. Horns of these cattle were medium; stumpy with length and diameter for male were 1 1.75±1.36 cm and 11.61±1.28 cm and female were 10.82±1.56 cm and 9.18±2.07 cm respectively. Ears of this cattle were moderately long, alert and slightly dropping with the average length of 16.15±2.87 cm. They possessed medium and fleshy dewlap, which were of

fair depth. Legs were clean and not so massive, well apart firmly and squarely set under the body. The tail was long and tapering finely to full switch with an average length of 92.29±4.29 cm. The hair coat was fine, short, straight and smooth. The average lactation length, lactation yield and daily milk yield of this type of cattle were 210.12±7.59 days, 805.74±36.52 liters, and 2.25±1.05 liters, respectively. The average age at maturity, gestation length, age at first calving, post partum heat period, calving interval and service per conception were 32.42±3.58 months, 283±7.53 days, 43.10±4.59 months, 44.47±3.47 days, 14.50±3.85 months and 1.60±0.58 respectively. This experiment was based on the field survey. So, further study is needed to be carried out on other characters like genetic characters of RCC in Bangladesh for their characterization, conservation and improvement.

Habib *et al.* (2009) showed that the present study was undertaken for the estimation of birth weight (bwt) and some non-genetic factors affecting on this trait in Red Chittagong Cattle (RCC). Sex, parity, season of birth, sire and year of birth were considered as non-genetic (fixed) effect on bwt. Data were collected on 106 RCC cattle from the progenies born in the Nucleus Herd of USDA funded RCC project under the department of Animal Breeding & Genetics at Bangladesh Agricultural University (BAU), Mymensingh during the period from 2005 to 2009. The mean bwt of RCC cattle was found 14.89 ± 0.224 kg. Male cattle were heavier (15.79 ± 0.286 kg) than female (13.96 ± 0.298 kg) as other breeds do. Bwt was significantly ($p<0.05$) affected by sex of the cattle. On the other hand sire, parity of cattle, season of birth and year of birth had no significant ($p>0.05$) effect on bwt.

Rahman *et al.* (2009) showed that the experiment was carried out for 28 days from August to September 2007 to investigate the efficacy of Ivermectin and Pineapple plants against ectoparasites in cattle. Effect of Ivermectin and Pineapple plants on certain blood parameters hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TEC) and erythrocyte sedimentation rate (ESR) were studied in cattle. A total of 15 cattle were examined for the presence of ticks by physical examination and were divided into three equal groups as, Group A (infected control group), Group B (treated with Pineapple) and Group C (treated with Ivermectin).

The therapeutic efficacy of Ivermectin was 100% against ectoparasites in cattle on day 7, 14, 21 and 28 after the treatment of Ivermectin. Ivermectin and Pineapple showed significant effectiveness at 7th, 14th, 21st and 28th day of post treatment. The effects on TEC, Hb level was increased significantly at 7th, 14th and 28th day of treatment and PCV level was increased significantly on 28th day of treatment. On the other hand indigenous medicinal plant Pineapple stem shown the efficacy of 68% at day 28 and body weight of Ivermectin and Pineapple stem treated cattle also increased. All the cattle after Ivermectin injection and Pineapple stem spray remained healthy, no adverse effect and cattle appetite increased, growth and coat color improved rapidly.

Demircan *et al.* (2007) studied that Purpose of this study was to determine effect of season on beef cattle performance and profitability in beef cattle farms in Afyon province. Data were obtained by conducting a questionnaire with 100 beef cattle farms selected by stratified random sampling method. Starting date on feed was assigned to seasons and, since the steers were fed an average for 202 days, an overlapping of seasons occurred. Thus, cattle started on feed in spring, autumn, summer and winter were finished in summer, spring, autumn and spring, respectively. Cattle started in spring, autumn, summer and winter were exposed to hot, cold and warm portions of the year, respectively, and were classified as hot, cold and warm season cattle. Cattle raised in cold season had lower average daily gain (ADG) than those in warm season ($P<0.05$). Feed efficiency of cattle raised in warm and hot season was better than those raised in winter ($P<0.05$). It was found that cattle raised in cold season had higher cost for 1 kg of live weight, live weight gain and carcass than other seasons ($P<0.05$). Gross profit, net profit and relative return of cattle raised in cold season was lower than other seasons ($P<0.05$). When average profit of all farms were considered, it was found that profit was not enough to sustain a farm household's living. In the study area cost and sale price of 1 kg carcass were close to each other thus discrepancy between these two prices defined, as profit margin was small.

Chandrawathani P *et al.* (2006) reported that study was conducted to evaluate the Anthelmintic Effect of Pineapple (*Ananus comosus*) On Nematode Parasites of Sheep. Twelve Santa Ines Cross Bred Sheep from a Government Farm were randomly selected and equally divided into control (n = 6) and treated groups (n =6). Faecal egg counts (FEC) using the modified McMaster technique and the FAMACHA score for assessing clinical anaemia were carried out daily and recorded for 6 weeks. At the end of the study all the animals were slaughtered and the total worm count (TWC) was done. The results of FEC showed that there was no significant difference between the control and treated group ($p = 0.081$). However, worm burden estimations showed that the number of parasites was significantly higher in the control group compared to the treated group ($p < 0.05$). This result indicated that feeding Pineapple had an effect on worm numbers in sheep, but was not reflected in their faecal egg counts. Further work is needed to reconfirm the effect of Pineapple on helminthes infections of sheep.

Silveira *et al.* (2005) studied that beef cattle production is suffering numerous difficulties in productive and economical terms in the state of Rio Grande do Sul. Expanding new markets, competition with other meats, external competition through imported Mercosul beef, new growth and slaughter processes bring new challenges to the Brazilian livestock production. In the case of Rio Grande do Sul, another factor to be considered is the competition with Central States in the internal market. Meat prices received by farmers in the last years stayed constant, but prices of the main production inputs are still rising. This scenery brought continues income decrease to the beef cattle farmers. As a result, many farmers are selling part of their lands or leaving beef cattle production. This paper aims to compare gross income and cash flows in three farms with different beef cattle systems during the years of 2003 and 2004, as well as analyzing the cost percentage of different inputs necessary to the production systems. The farms are located in different regions: Campanha, Depressão Central and Fronteira Oeste, representing important agricultural production regions in the in Rio Grande do Sul state. The results show that without technological modernization in beef cattle production systems and integration with

agriculture, it becomes difficult to re-munerate all the production factors. Planning allows a better income distribution, reducing the months of negative cash flow. However, the low profit per area demonstrates the gravity of the economical situation of beef cattle farmers in Rio Grande do Sul.

Rainey *et al.* (2004) conducted that three experiments were conducted to test the effects of animal age (cows vs. cattle) and barley processing method (whole vs. rolled) on rate and efficiency of gain and diet digestibility when barley was fed as a supplement to medium quality grass hay. A fourth experiment was conducted to test the effects of differences in diet composition on ADG and carcass characteristics in early-weaned cattle. No age x processing interactions ($P > 0.05$) were detected for OM, N, ADF or NDF digestibilities. Diet OM and N digestibilities were greater ($P < 0.01$) when consumed by cattle compared to Cows, but ADF and NDF digestibilities were similar between ages. A processing method x age interaction was measured ($P < 0.05$) for starch digestibility. Rolling the barley dramatically improved starch digestibility when fed to cows (71.4% vs. 23.3% for rolled vs. whole). For experiment two, animals fed the hay only diet had similar ($P > 0.05$) rates and efficiencies of gain as diets supplemented with barley. Barley processing had no effect ($P > 0.05$) on rate or efficiency of gain for cows or cattle. Cows were less efficient than cattle (12.6 vs. 7.6) when fed similarly formulated diets. Unlike experiment 1, no differences were measured for digestibility of OM, N, ADF, NDF, or starch due to animal age or grain processing method. For experiment 4, cattle fed barley gained faster (1.29 kg·d-1 vs. 0.99 kg·d-1; $P = 0.002$) and had higher marbling scores (4.44 vs. 3.31 %EEF; $P = 0.002$) compared to cattle fed wheat midds during the first 34 d after weaning. However, gains during the 90 d after weanining were no significant (1.36 vs. 1.24 kg·d-1 for barley and midds respectively). Calculated final live weights and marbling scores after 217 d of consuming a common finishing ration were similar for barley-fed cattle compared to wheat midds-fed cattle. Results suggest processing of barley may be of greater value for mature cows compared to younger cattle, and barley had a greater effect on changing cattle body composition than wheat midds. Cost·kg-1 gain was similar for whole vs. rolled barley diets.

Miller *et al.* (2004) studied that One hundred-sixteen multiparous Angus × Gelbvieh cows (initial BW = 571 ± 63 kg, BCS = 5.4 ± 0.7) were blocked by BW, assigned to one of 18 pens, and received one of two dietary treatments from d-31 to 125 of gestation (Exp. 1). Control (C) cows were fed native grass hay fortified with vitamins and minerals at recommendations for a mature cow to gain 0.72 kg/d for the first 120 d of gestation. Nutrient restricted (NR) cows were fed one half C minerals and vitamins, and millet straw at 68.1% of NE_m requirements. Along with BW and BCS, ultrasound measurements of ribeye area (REA), 12th rib fat thickness at the 12^{th} rib (BF), and percent i.m. Fat of the LM (IMF) were collected every 14 d. In Exp. 2, 96 cows from Exp. 1 were re-blocked according to BW and BCS and assigned to one of 16 pens. Control cows continued to be fed as in Exp. 1, while NR cows were realimented with the target of achieving BCS similar to C cows by 60-d prepartum. Body weight and BCS were measured every 14 d, whereas REA, BF, and IMF were measured every 28 d. A subset of cows from Exp. 1 (n = 20) and Exp. 2 (n = 10) were harvested to determine correlations between ultrasound and carcass measurements of REA ($r = 0.49$, $P = 0.006$), BF ($r = 0.85$, $P < 0.001$), and IMF ($r = 0.69$, $P < 0.001$). In Exp. 1, treatment × day of sampling interactions were noted ($P \leq 0.001$) for all variables. Body weight, BCS, BF, IMF, and REA were reduced ($P < 0.05$) by d-59, 45, 59, 73, and 73 of gestation, respectively. In Exp. 2, BW and BF remained less ($P < 0.002$) for NR than C cows throughout the realimentation period. Cow BCS and REA were lower ($P \leq 0.03$) for NR versus C cows until d-164 of gestation, but were similar ($P = 0.11$ and $P = 0.58$, respectively) by d-192 of gestation. Ultrasound may be a useful technology to predict changes in body composition associated with a beef cow's nutritional plane.

Schwalbach *et al.* (2003) studied that the aim of this experiment was to evaluate the efficacy of Pineapple stem extract (10% water solution) for tick control in goats in the Kilimanjaro area of Tanzania. Eighteen Small East African (SEA) and Toggenburg (TB) female goat kids between 2.5 and 4 months of age were used in the trial. Goats were divided into two similar groups according to age, body weight and breed composition. Ten treated kids (n = 6 SEA and n = 4 TB goats) and eight

controls (n = 4 SEA and n = 4 TB goats) were housed in separate pens for shelter at night, but grazed/browsed together during the day on natural pastures infested with adult and immature ticks. The Pineapple stem -treated group was hand sprayed weekly (spot treatment) with a 10% aqueous solution of Pineapple stem extract to the following regions: perineum, udder, ears and sternum at a rate of 10 mL/kg body weight. The control animals were sprayed weekly with water (10 mL/kg body weight) to the same sites. Pineapple stem -treated goat kids recorded significantly lower tick numbers than the control animals, with significantly lower tick numbers and tick bite abscesses in the indigenous SEA goats compared to the goats of the exotic TB breed. Significantly higher tick infestations were recorded on both breeds of goats during the summer rainy season. It was concluded that Pineapple stem extract is effective in controlling ticks in goats. Further studies are necessary to fully understand the effect of Pineapple on ticks.

Rivera *et al.* (2002) three experiments were conducted to examine the effect of dietary vitamin E on receiving performance and health and on finishing performance of beef cattle. One hundred twenty beef steers (Exp. 1; initial BW =173 kg) and 200 beef heifers (Exp. 2; initial BW =204 kg) were assigned randomly to one of three treatment diets formulated to supply 285, 570, or 1,140

IU/animal daily of supplemental vitamin E during the receiving period. Average daily gain, gain:feed, and DMI were calculated every 14 d, with pen as the experimental unit. Morbidity and retreatment data were analyzed using a nonparametric procedure. After the receiving period, cattle were assigned to a grazing period followed by a finishing program and fed until slaughter. In Exp. 3, 17 beef steers were used to evaluate effects of the same three vitamin E levels on humoral immune response to an ovalbumin vaccine given on d 0 and 14. Jugular blood samples were collected on d 0, 7, 14, and 21. In Exp. 1, vitamin E did not affect ($P \geq 0.10$) ADG, DMI, or gain:feed for d 0 to 14, 14 to 28, or 0 to 28. No

Effects were noted for percentage of morbidity; however, cattle receiving 1,140 IU/d had a numerically ($P = 0.15$) lower incidence of retreatment. During the 91-d finish-

ing phase, a quadratic effect ($P \leq 0.08$) was noted for DMI, ADG, backfat thickness, longissimus muscle area, and yield grade. In Exp. 2, a tendency for a linear ($P = 0.10$) increase in ADG was observed for the first 14 d of receiving; however, ADG decreased linearly ($P = 0.06$) with vitamin E concentration thereafter. For the 28-d period, ADG and DMI did not differ among treatments,

But gain:feed decreased linearly ($P \leq 0.05$) for d 14 to 28 and for d 0 to 28. No effects on percentage morbidity were noted in Exp. 2, and no differences were detected for ADG, gain:feed, or DMI for the 98-d finishing period. There was a linear increase in yield grade ($P \leq 0.05$) and a linear ($P \leq 0.08$) decrease in longissimus muscle area with increasing vitamin E. Heifers receiving 570

IU of vitamin E during the receiving period tended to have a higher ($P \leq 0.09$) dressing percentage at slaughter.In Exp. 3, no significant differences were detected

In serum IgG titers to ovalbumin on d 0, 7 or 14; however, on d 21, a linear increase ($P=0.07$) in serum IgG titers was noted with supplemental vitamin E. Supplemental vitamin E had limited effects on performance; however, effects on humoral immune response and recovery from respiratory disease warrant further research.

Louis *et al.* (2002) studied that Ivermectin (22, 23-dihydroavermectin B1) is a broad-spectrum antiparasitic drug that was introduced for the control of parasitic worms and lice in sheep and cattle. This review summarizes what is known about the impact of ivermectin in pastures and catchments and also on freshwater systems. Differing results have been obtained for the effects on decomposition of dung from ivermectin-treated cattle, although other experiments have shown adverse effects on growth of earthworms. There were likely to be risks to sediment-dwelling invertebrates where farmed salmon had been treated with ivermectin to control sea lice. In laboratory tests, freshwater fish appeared to have low sensitivity to ivermectin. In view of the lack of published information about environmental effects of use of avermectins and about endocrine-disrupting chemicals in detergents used in

dairy operations, it is recommended that monitoring of dairy discharges for residues of such chemicals should be undertaken.

Auchtung *et al.* (2001) showed that in dairy cattle, increased circulating growth hormone has been associated with selection for greater milk yield. This study tested the hypothesis that beef cows divergently selected for milk production would have differing GH responses to a challenge dose of GHRH. Growth hormone response to a challenge of GHRH was measured in 36 Angus-sired cows ranging from 6 to 10 yr of age. The cows were classified as high milking (n =16) or low milking (n =20), on the basis of their sires' milk EPD. Mean milk EPD (in kilograms) was 16.6 and −14.4 for high and low milking cows, respectively. Milk production was estimated by the weigh-suckle-weigh procedure. Blood samples were taken immediately before and 10 min after a clearance dose of 4.5 g of GHRH/100 kg BW (injected i.v.) and, 3 h later, immediately before and 10 min after a challenge Dose of either 1.5 or 4.5 g of GHRH/100 kg BW. Each animal received both challenge doses, and the doses were randomly assigned across 2 d of blood collection. Serum concentrations of GH and IGF-I were measured by RIA. Serum IGF-I was measured in the baseline blood sample on d 1 of blood collection. A positive relationship (r =0.35; P =0.03) was observed between the cows' rankings for each dose of GHRH; that is, high responders to the low dose were high responders to the high dose. Growth hormone response to the 4.5 g /100 kg BW challenge dose of GHRH was positively related to sire milk EPD (R2 =0.09; P =0.03). Response of GH to the 1.5 g GHRH/100 kg BW challenge dose also tended to be related (P =0.08) to sire milk EPD of high milking cows. In addition, IGF-I concentrations of high milking cows were inversely related (R2 = 0.24; P = 0.04) to sire milk EPD. Growth hormone response to GHRH challenge may have potential as an additional tool in the evaluation of milk production in beef cattle.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Collection and processing of plant materials

Pineapple stems were selected for evaluation for its effectiveness against endoparasites in cattle. Mature and disease free Pineapple stems were collected from Hajee Mohammad Danesh Science and Technology (HSTU) campus. After collection, stem were washed in running tap water and cut into small pieces.

3.1.1 Preparation of fresh juice

Fresh stem were cut into small pieces and warm water was added at 1:10 ratio in a kitchen blender. Then juice was made by blending the leaves for 2-3 minutes and crude extract was stored in a refrigerator at 4°C.

3.1.2 Preparation of dust of Stem

For the preparation of dust, the stem were dried in sun for 10 days and followed by oven at 55-60°C for 2 days. The dried stem were pulverized with a blender. A 25 (unit) mesh diameter sieve was used to obtain the fine dust, after then dust was preserved in airtight plastic container until they were directly used for screening and preparation of alcoholic extract.

3.1.3 Preparation of alcohol treated stem dust

Stem dust was used for preparation of plant extract. 100 gm dust were taken in a 500 ml beaker and separately mixed with 1 liter of ethyl alcohol. Then the mixture was stirred for 30 minutes by a magnetic stirrer (6000 rpm) and left stand for next 24 hrs. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50°C for ethanol. After the evaporation of solvent from filtrate, the condensed extracts were

preserved in tightly corked-labeled bottle and stored in a refrigerator at 4°C. During the experimental period different concentrations of the extract, viz 5, 10, 20 and 40 mg/ml, were used in normal saline.

3.2 Experimental trial for comparative efficacy of Pineapple stems extract and a patent drug for anthelmintic effects against Endoparasites in cattle

3.2.1 Collection and management of Cattle

Fifteen (15) cattle were randomly selected from Asia Dairy Farm, Baroil, Gopalgonj, Dinajpur. Fecal eggs of parasites were counted .On the basis of fecal egg count, the cattle found positive with eggs of *Ascaridia sp.* were separated. From the positive cases, 15 cattle were again randomly selected for the experimental trial. The finally selected 15 cattle were allowed to acclimatize for 7 days in the experimental shed. The body weights (bd. wt.) of assigned cattle were recorded. During acclimatization, the cattle were supplied recommended feed and water.

3.2.2 Fecal egg counts

For determinations of infectivity, fecal samples were collected and eggs were counted by egg counting improved McMaster as well as direct smear method.

3.2.3. Egg counting improved McMaster method

Materials: Slides, cover slips, glass-bead, 120 ml bottle, wire-mesh screen (Aperture 0.15 mm), stirrer, centrifuge machine, Pasteur pipette and McMaster counting chamber.

Methodology: About 50 glass balls were placed in a 120 ml bottle and added 45 ml of warm water. From collected sample 5 gm of faces were weighed and put in bottle. Stopper was fitted to the bottle and shaken until all the fecal material was broken down. The mixture was poured through a wire mesh screen with an aperture of 0.15 mm and the strained fluid was taken in a bowl. The debris left was discarded off the screen. The strained fluid was stirred and a sample was poured into a centrifuge tube,

within 1 cm of the top. The tube was shaken until the sediment was loosened and formed a homogeneous sludge at the bottom of the tube. The tube was filled with saturated salt solution to the same level as before. The contents of the tube were thoroughly mixed by inverting it five or six times with the thumb over the end and sufficient of the fluid was immediately withdrawn with a Pasteur pipette and content was allowed to run into one chamber of the counting slide carefully. After further mixing, a second sample was withdrawn and allowed to run into the other chamber. All the eggs were counted in the two separate central square. The number of eggs per gram of feces were calculated and recorded by multiplying the total eggs in the two squares by 50.

3.2.4. Direct smear method

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

Methodology: A small quantity of feces was placed on slides with the help of tooth-picks, mixed with a drop of water, spread out, covered with a slip and examined directly under microscope with low power. At least three slides from each fecal sample were examined.

At least 5gm of feces were collected. Samples in which eggs of ascariasis found.

3.3. Selection and collection of drugs/chemicals

Ivermectin and Pineapple (*Ananus comosus*) stem extract were selected. Pineapple (*Ananus comosus*) were collected from "Medicinal plants garden" Department of Physiology and Pharmacology, HSTU, Dinajpur. Pineapple stem (10%) was prepared from processed dusts of the stem in distilled water. Briefly, 10gm of stem dusts were mixed into 100 ml distilled water, then the mixture was stirred for 30 minutes by the same magnetic stirrer at 600 rpm as done earlier and left for 24 hours. The mixture was then filtered through filter paper (Whatman 42). The filtered

fluid was the 10% aqueous extract of Pineapple stem. Ivermectin was purchased from local market.

Experimental Layout

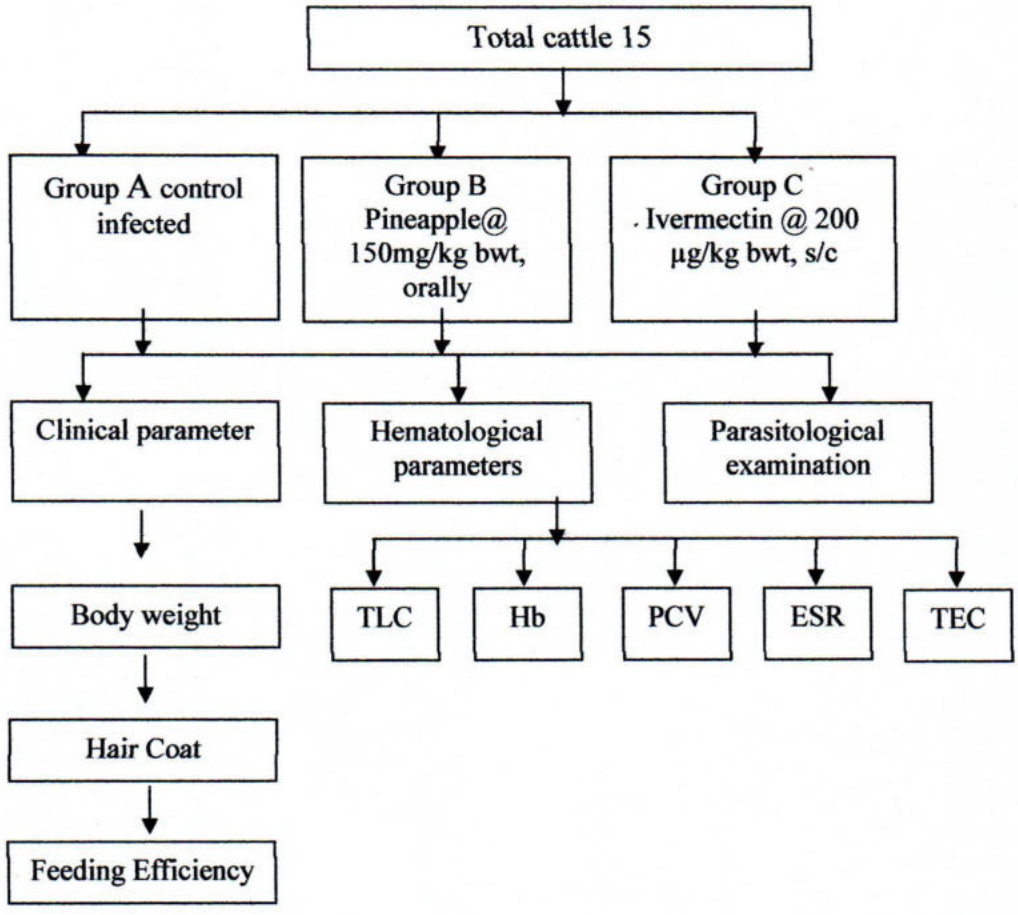

Fig. 3.1 Layout of the experiment

3.4 Evaluation of the comparative efficacy of one Ivermectin and Pineapple (*Ananus comosus*) stem extract against ascariasis in cattle

3.4.1 Experimental design

Fifteen (15) infected cattle were randomly divided into 3 equal groups namely A, B, and C

Group 'A': was kept as control without giving any treatment.

Group 'B': was treated with Pineapple (*Ananus comosus*) stem extract orally @ 150mg/kg bwt for consecutive seven days.

Group 'C': was treated with Ivermectin @ 300 mg/kg bwt, sc as a single dose

All the cattle of treated and control groups were closely observed for 28 days after treatment and following parameter were studied:

3.4.2 Clinical examination

- All cattle of both the treated and control groups were closely observed for any illness and behaviours.
- The effect of the Pineapple (*Ananus comosus*) stem extract, anthelmintic Ivermectin on bd. wt, feed consumption and water consumption were recorded before and during the treated period.
- The weight of each cattle was taken in the morning, in noon and afternoon. The average of these three weights were calculated and recorded.

3.4.3 Hematological parameters

Blood samples were collected from Jugular vein of cattle of both control and treated groups at pre-treatment and during treatment (28 days) period at 7 days interval to study the effect of the Pineapple (*Ananus comosus*) extract, anthelmintic Ivermectin on the following hematological parameters (plate 8) :

(a) Total erythrocyte count (TEC)

(b) Hemoglobin estimation (Hb)

(c) Packed cell volume (PCV)

(d) Erythrocyte sedimentation rate (ESR)

(e) Total leukocyte count (TLC)

3.4.3.a. Determination of Total erythrocyte count (TEC)

Total erythrocyte count was done following the method described. 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.

3.4.3.b. 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 hematin 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 g %. The above procedure was matched by the Hellige hemometer method as described.

3.4.3.c. 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 hematocrite 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.

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

3.4.3.d. Determination of erythrocyte sedimentation rate (ESR)

The fresh anticoagulant (Heparin) 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.

3.4.3.e. Determination of Total leukocyte count (TLC)

The principles involved in enumeration of Total Leukocyte Count were almost same to those of erythrocytes. Here the leukocyte diluting fluid was N/10 HCI solution. Well mixed blood was drawn up to the 0.5 mark of white blood cell pipette. The diluting fluid was filled up to the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes. 2-3 drops of content were discarded and counting chamber was then filled in the same way as in the red blood cell count. The counting chamber was placed under the microscope and examined under low power objective (10x). The leukocytes in the 4 large squares (each 1 square mm.) of the counting chamber were counted × 50 and expressed the result in thousand per cu. mm.

3.4.4 Statistical analysis

Statistical analyses were carried out by Statistical package for social science (SPPS) using F test. The data were analyzed statistically between control and treated groups of calvs by using paired sample t-test (Mostafa, 1989).

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 Comparative efficacy of patent drug Ivermectin and Pineapple stem on the body weight gain of cattle

4.1.1 Clinical Examination

4.1.2 Effect on Body Weight

The effect of patent drugs Ivermectin and one indigenous medicinal plant namely Pineapple (*Ananus comosus*) leaves on body weight were observed on day 7th, 14th, 21st and 28th respectively. Body weight of each group of cattle prior to treatment was taken and live weight gain of each group of cattle on 7th, 14th, 21st and 28th days was calculated in gram is shown in the Table 4.1. The increased values of body weight in cattle of the treated groups were not significant on Day 7 and Day 14 but that were highly significant ($p<0.01$) on Day 21 compared to control.

Table 4.1: Effects of Ivermectin pour on and Pineapple stem extract on body weight (gm) in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment Body Weight	Post treatment body weight			
				7th day	14th day	21st day	28th day
A	Control (Infected)		89.4	89	89	89.8	90
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	100.5	100	103	104.5*	106.01
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	120.21	120	121.5	121.75*	125

4.1.3. Fecal egg count

The results of the efficacy of Pineapple stem and Ivermctin are shown in the Table 4.2 Reduction of EPG count was found on 14th and 21st day in the group of cattle of B, C and on the other hand EPG count was found increased or same in the groups of A (control).

Table 4.2: Effects of Pineapple stem and Ivermectin on Nematodes egg in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment EPG	Post treatment			
				7th day	14th day	21st day	28th day
A	Control (Infected)		1305	1340	1370	1275	1390
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	1010	500	650	250*	150
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	1110	00	00	00	00

* Significantly increased ($p<0.05$)

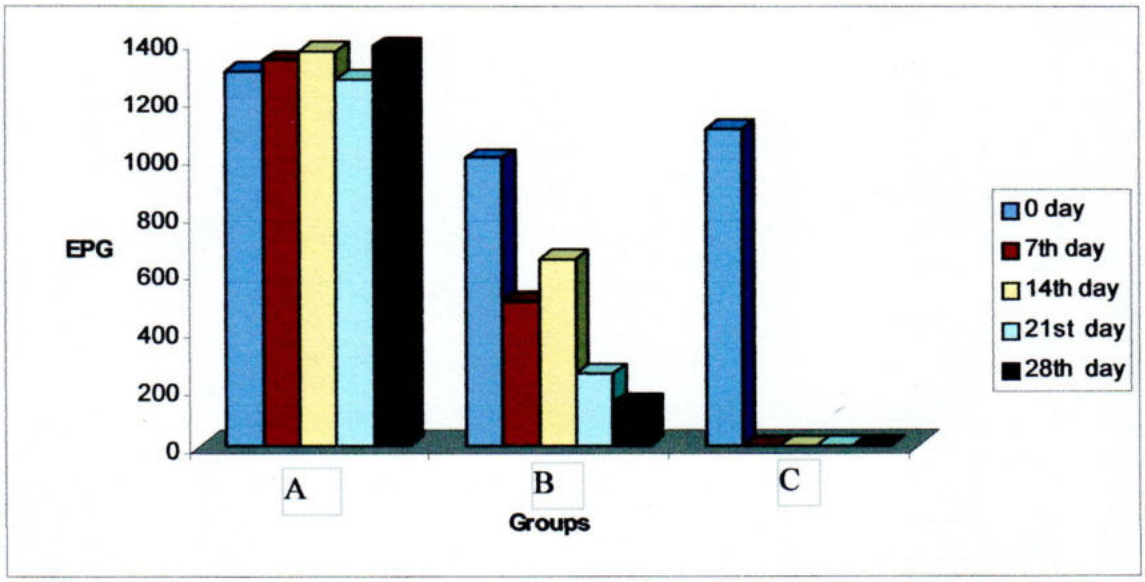

Fig: 2. Effects of Pineapple stem and Ivermectin subcutaneously in Nematodes egg in cattle

4.2. Hematological parameters

4.2.1 Effect on total erythrocyte count (TEC)

The mean values of TEC increased considerably in cattle treated with the Ivermectin and Pineapple stem extract on day 21 compared to that on day 0 but that were increased significantly ($p<0.01$ in Group B and C) on Day 21 in all the treated groups compared to control. The values were variable among all samplings in control cattle.

Table 4.3: Effects of Pineapple stem extract and Ivermectin on TEC (million/cu mm) in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment	Post treatment			
				7th day	14th day	21st day	28th day
A	Control (Infected)		8.05	8.04	8.10	7.09	6.98
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	8.11	8.16	8.23	8.39*	8.48
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	8.12	8.19	8.30	8.40*	9.05

* Significantly increased ($p<0.05$)

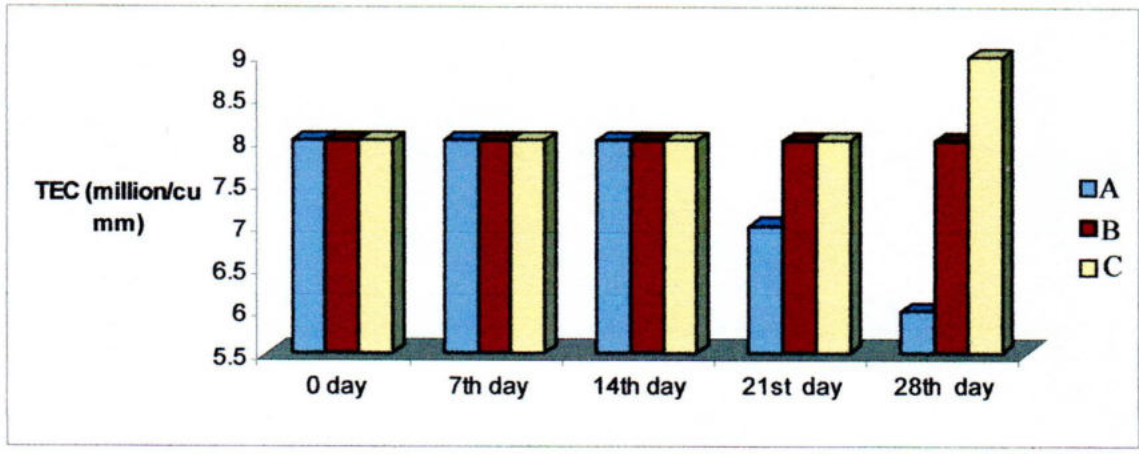

Fig: 3 Effects of Pineapple stem extract and Ivermectin on TEC (million/cu mm) in cattle

4.2.2 Effect on Hb Estimation

The mean values of Hb were increased from Day 7 up to Day 21 in cattle of Pineapple stem extract and Ivermectin treated groups, but that were decreased on the same sampling days in control cattle. The increased values of Group B and Group C were statistically significant on day 21 compared to control.

Table 4.4: Effects of Pineapple stem extract and Ivermectin on Hb% in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment	Post treatment			
				7^{th} day	14^{th} day	21^{st} day	28^{th} day
A	Control (Infected)		7.62	7.46	7.21	7.09	6.95
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	8.25	8.25	8.35	8.45*	8.75
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	8.10	8.70	8.90	9.00*	9.20

* Significantly increased ($p<0.05$)

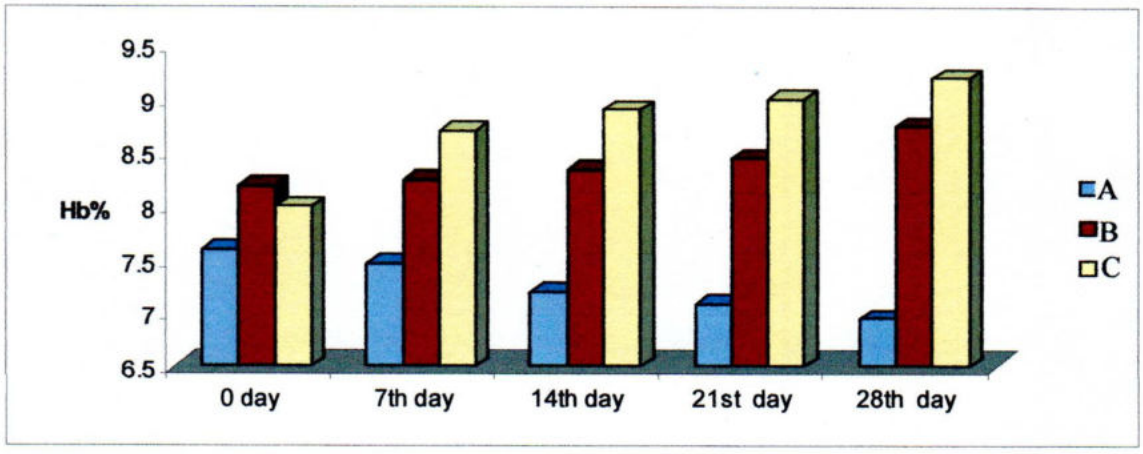

Fig: 4. Effects of Pineapple stem extract and Ivermectin on Hb% in cattle

4.2.3 Effect on Packed cell volume (PCV)

The mean values of PCV increased in the Pineapple stem extract and Ivermectin treated cattle from starting of treatments up to the end of the treatments, but that decreased in control cattle in the same way. The increased values in cattle of treated groups were not significant on day 7 and day 14 but that were highly significant ($p<0.01$) on day 21 compared to control.

Table 4.5: Effects of Pineapple stem and Ivermectin on PCV (% 30 minutes) in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment	Post treatment			
				7^{th} day	14^{th} day	21^{st} day	28^{th} day
A	Control (Infected)		30.65	30.40	30.25	30.15	29.10
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	30.50	30.45	30.50	30.65	30.75*
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	30.30	30.38	30.47	30.68	30.80*

* Significantly increased ($p<0.05$)

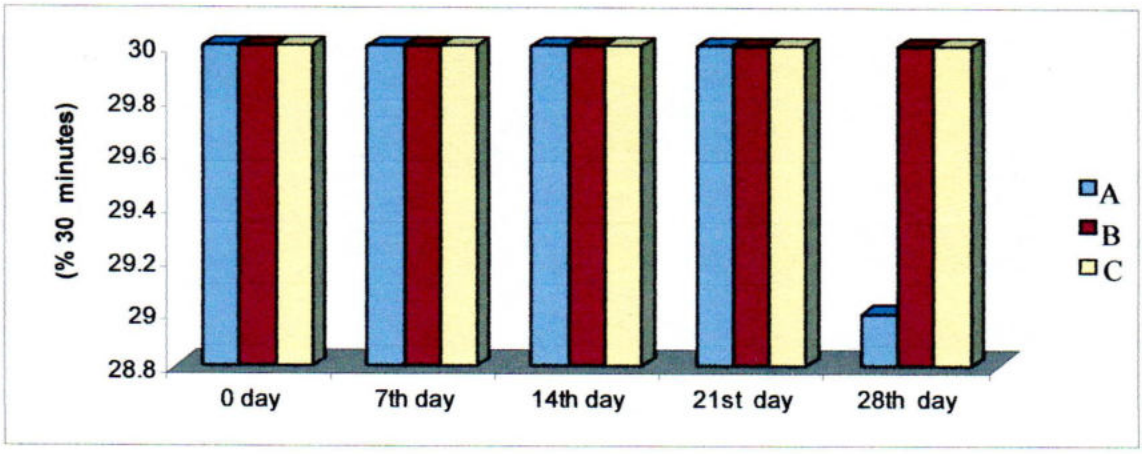

Fig: 5. Effects of Pineapple stem extract and Ivermectin on PCV (% 30 minutes) in cattle

4.2.4. Effect on erythrocyte sedimentation rate (ESR)

The mean values of ESR decreased in the Pineapple stem extract and Ivermectin treated cattle starting from Day 7 and continued up to the end of the treatments, but that increased in control group cattle in the similar manner. The difference of the values on Day 21 was highly significant ($p<0.01$) in both the treated cattle compared to control.

Table 4.6 Effect of Pineapple stem extract and Ivermectin on ESR (mm/1st hour) in cattle

Group n = 5	Drug with dose	Route	Pre-Treatment	Post treatment			
				7th day	14th day	21st day	28th day
A	Control (Infected)		1.03	1.09	1.40	1.85	2.00
B	Pineapple @ 150mg/kg bwt for consecutive days	Orally	0.30	0.25	0.18	0.08	0.05
C	Ivermectin @ 200 µg/kg bwt as a single dose	S/C	0.28	0.19	0.10	0.05	0.02

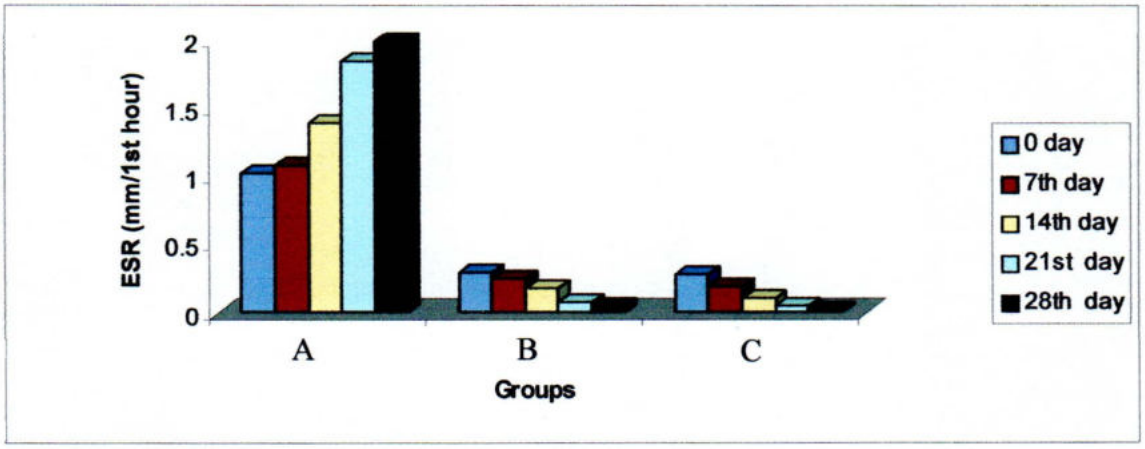

Fig: 6. Effect of Pineapple stem extract and Ivermectin on ESR (mm/1st hour) in cattle

Picture 3: Pineapple stem

Picture 4: Collection of pineapple stem

Picture 5: Pineapple stem and Ivermectin

Picture 6: Ivermectin

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

When efficacy and persistent efficacy are assessed in domesticated ruminants, control groups and worm burdens are preferably used due to more accuracy (Vercruysse *et al*, 2001). However, in ruminants, where no losses are allowed, FECR tests are easy to apply and give sufficient information. Different formulae are used to calculate egg reduction, and whereas in larger herds of domesticated ruminants the use of geometric means is common, arithmetic means are used more in small herds of ruminants (Young *et al*., 2000). In this study, no significant differences in efficacy were observed when different methods of calculation were used.

These results compare favourably with those of McPherson (1995). Pineapple (*Ananus comosus*) has ability to suppress internal parasites. Pineapple has also significants on blood parameters.

Finally, Cattle gains better body weight in-group B and C than control group A. But further therapeutic study needed for more confermation.

CHAPTER VI

SUMMARY AND CONCLUSION

In this experiment, y Pineapple extract (*Ananus comosus*) were studied in terms of anti-endoparasitic infestation and better body weight gain in cattle and there is no side effects.

The experiment was conducted in the Department of Physiology and Pharmacology, Faculty of Veterinary Science, HSTU, Dinajpur. Fifteen (15) healthy cattle of aged between were divided into three equal groups (n=10) to carry out this research work.

Keeping one group as normal control rest groups were subjected to treated with Pineapple (*Ananus comosus*) and Ivermectin. The blood parameters and body weights were measured in all groups of cattle. Pineapple stem powder@ 100mg/kg bwt and Ivermectin @ 200 µg/kg bwt, sc. Weekly observations were recorded for live body weight, weekly gain in weight, weekly feed consumption, feed efficiency and blood parameters of cattle for six months. All the treatment groups B (106.01) and C (125) recorded significantly higher means for live body weight than that of control A (90) group.

All the groups of cattle were observed for 30 days of treatment.

In this research work, the continuous treatment with extract of Pineapple stems (*Ananus comosus*) produced a significant reduction ($p<0.01$) of the endoparasites and Ivermectin also reduced internal parasites. In Pineapple (*Ananus comosus*) there is no side effects and body gain was significant that groups recorded significantly higher means for live body weight than that of control A (90) group.

In Bangladesh, only few trials have been performed to evaluate the medicinal valu
of Pineapple (*Ananus comosus*) stem. We did the work in short-term basis (onl
28days) and modern equipments were also not available. To establish Pineapple
(*Ananus comosus*) stem and as an alternative anthelmintics and increase body weitht
further therapeutic study is needed.

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