"Comparative efficacy of extracts of some medicinal mushrooms against coccidiosis in broilers"

DISSERTATION

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by

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M.sc

UNDER THE SUPERVISION OF

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Certificate

This is to certify that the dissertation entitled **"Comparative efficacy of extracts of some medicinal mushrooms against coccidiosis in broilers"** submitted to the University of Kashmir for the award of the Degree of **Master of Philosophy in Zoology,** is the original research work of **Ms. Shazia Ahad,** a bonafide M. Phil. Research Scholar of the Department, carried out under my supervision. The dissertation has not been submitted to this University or to some other University so far and is submitted for the first time. It is further certified that this dissertation is fit for submission for the degree of Masters of Philosophy (M. Phil.) in Zoology and the candidate has fulfilled all the statutory requirements for the completion of the M. Phil. Programme.

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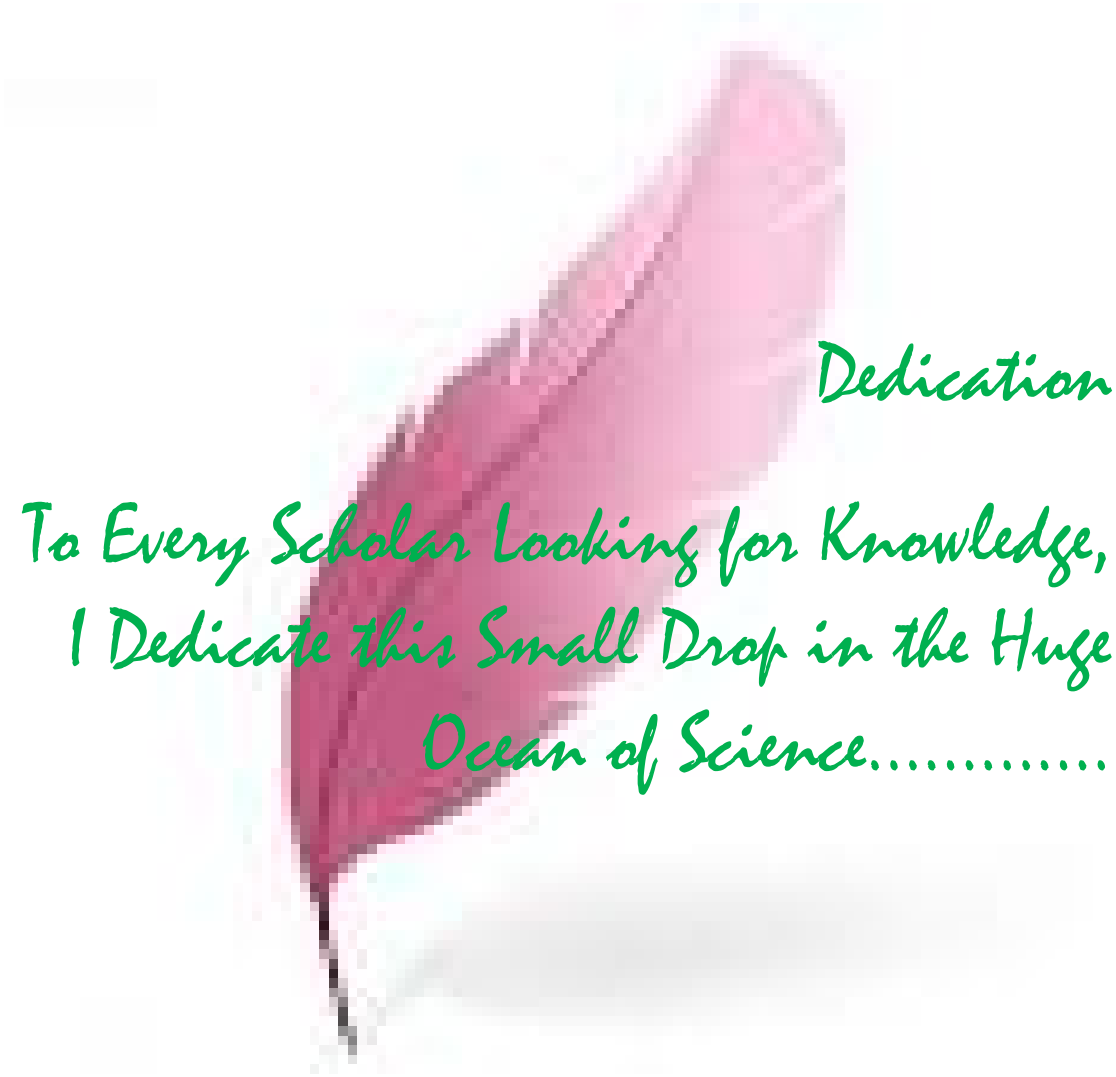

Dedication

To Every Scholar Looking for Knowledge,
I Dedicate this Small Drop in the Huge
Ocean of Science.....

ACKNOWLEDGEMENTS

All the praises and thanks are for ALMIGHTY ALLAH, the most merciful and compassionate, the most gracious and beneficent, whose bounteous blessings with health, thought, talented teachers, helping friends to make contribution to already existing ocean of knowledge and in the accomplishment of this manuscript.

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Shazia Ahad

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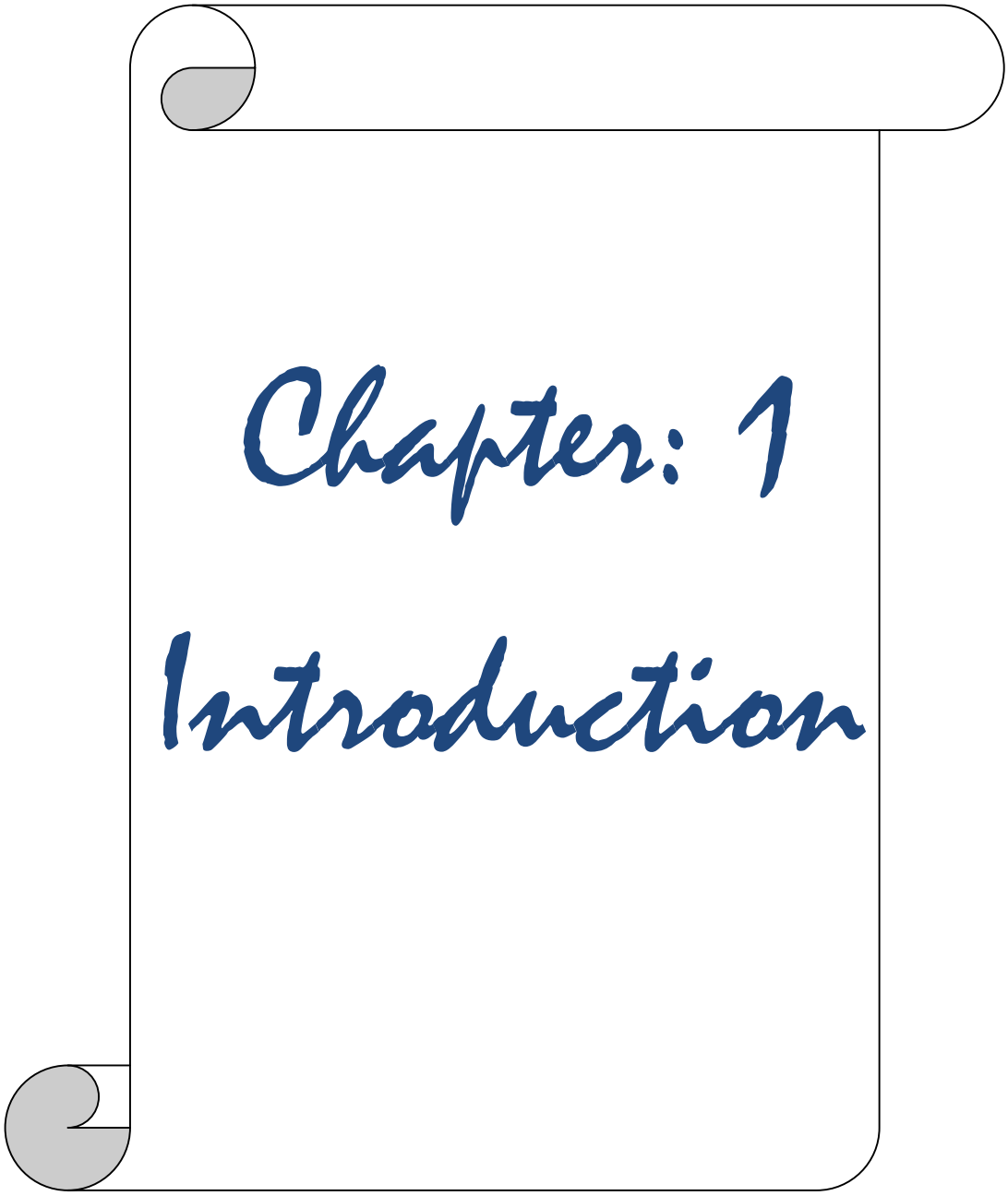
List of Abbreviations

^{0}C	Degree Centigrade
%	Percentage
@	At the rate of
BW	Body weight
Cm	Centimetre
DFI	Daily feed intake
DWG	Daily body weight gain
DWG	Daily body weight gain
EARO	Ethopian Agricultural Research Organisation
EPG	Eggs Per Gram
Exp	Experiment
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
Fig	Figure
FMG	Fungus Myceliated Grain
g	Gram
GDP	Gross Domestic Production
Hb	Haemoglobin
hr	Hour
i.e.	That is
Kcal	Kilo calories
KDa	Kilo Dalton
Kg	Kilogram
Km	Kilometre
mg	Milligram
MIC	Minimal inhibitory concentration
MIC	Minimal inhibitory concentration
Ml	Millilitre

mm	Millimetre
NK	Natural killer cells
NRC	National Research Council
OPG	Oocyst per gram faeces
PC	Post Challenge
PCV	Packed cell volume
PI	Post inoculation
ppm	Parts Per Million
RBC	Red blood cells
rpm	Rotations Per Minute
SD	Standard deviation
SP/SPP	Species (singular/ plural)
SSPS	Small Scale Production System
WBC	White blood cells
Wt	Weight


Chapter: 1
Introduction

Poultry production in its general term includes the production of domesticated birds such as chicken, turkeys, ducks, geese and others, which are mainly kept for production of egg and meat. Among these, chicken are the most important species adapted globally to different ecological condition where humans live and contribute significantly in supplying animal protein to improve human nutrition (EARO, 2000). Poultry birds are kept in backyards or commercial production systems in most areas of the world. Compared to a number of other livestock species, fewer social and religious taboos are related to the production, marketing, and consumption of poultry products. For these reasons, poultry products have become one of the most important protein sources for people throughout the world. The total number of poultry birds in the world has been estimated by the Food and Agriculture Organization of the United Nations (FAO) as of 14,718 million, with 1,125 million distributed throughout the African 1,520 million in South America and 6,752 million in Asia, 93 million in Oceania, 3,384 million in North America and 1,844 million in Europe (Ander and Jorgen 1998). During the last two decades the growth of poultry industry has been remarkable and India presently holds third position in the world in terms of egg production and poultry meat production has reached about 2.2 million tons per year. It is evident that the poultry industry is not

only playing a great role for the nutritional security of the country, but also provides working opportunity to a sizeable population of the world. About three million people in India are directly linked to the poultry industry which in turn contributing to the national economy to the extent of US $5.7 billion annually (Tewari and Maharana, 2011). Moreover, poultry in many parts of the modern world is considered not only the chief source of protein of animal origin but also of high quality human food. The production of meat-type poultry has greatly expanded over the past several decades. The commercial broiler industry has evolved from backyard flocks into an ingenious mass food production system. The primary focus of the commercial broiler industry is to maximize profits by promoting maximal yield and maintaining the health of the bird. Any hindrance to bird health will decrease profitability. Improvements in technology relating to vaccines or nutrition could save companies money and allow the industry to operate more efficiently by increasing revenue and decreasing overall costs. One of the main expenses faced by the industry is loss associated with poultry diseases, including costs of vaccination, prevention, treatment, reduction in weight gains, and mortality.

Diseases result when normal body functions are impaired, and the degree of impairment determines the severity of the disease. It may result from the consequences of harmful actions of infectious and parasitic agents, or it may be caused by injury or physical stress with which the bird cannot cope. Disease may also occur as the result of a deficiency of a vital nutrient or the ingestion of a toxic substance. Diseases caused by infectious and parasitic agents are frequently complex and depend upon characteristics of the host, agent, and environmental conditions on the farm. A disease resulting from parasitism depends on the number, type, and virulence of the parasite, the route of entry to the body, the defense status and capabilities of the host. Parasites live on, in or around chickens. They can cause damage directly by disturbing the chickens and affecting their growth and egg production. They can also spread certain diseases, parasites can reside inside the chicken (internal parasites e.g. worms) or on the outside of the chicken. External parasites include lice, mites, ticks, fleas and flies. Internal parasites can be classified into several types based on their body types, life cycle, and damage to their hosts. Internal parasites of poultry include roundworms or Nematodes, tapeworms or

Cestodes, flukes or Trematodes, and Protozoa including *Eimeria* spp., *Cryptosporidia* spp., *Histomonas* spp. (Blackhead), *Trichomonas* spp. and other blood and tissue protozoans. A parasite obtains its nourishment from another organism, where it cannot live independently; these include species of coccidia that causes the poultry industry to suffer a considerable economic loss, especially in the production of broilers. Although most of the diseases of infectious origin affecting the poultry industry have been controlled successfully using protective vaccines, coccidiosis caused by several *Eimera* spp. is still considered as the most challenging deterrent for poultry development.

1.1. Coccidiosis

Coccidiosis is the most important protozoan disease affecting the poultry industry worldwide. It has been documented that coccidiosis is the most consistently reported health problem in poultry (Biggs, 1982; Williams, 1999). In our country, it is consider a serious problem causing huge economic loss to poultry industry, especially in the production of broiler chicken. Over the past 100 years, much research has persisted on coccidiosis because of its significance in the animal industry. In all parts of the world where confinement rearing is practiced, coccidiosis represents a major disease problem demanding the attention of poultry producers, feed manufactures, and poultry disease experts (Reid, 1978). Coccidiosis is believed to be a commonest depreciator or even a potential killer of our poultry. It is a parasitic disease of poultry caused by microscopic protozoan-type parasite called *Eimeria.* The disease is characterized by an invasion of the intestinal wall by parasite. This parasite lives and multiplies in the intestinal tract and causes tissue damage. The parasite then undergoes several stages of growth, during which there may be intense damage to the mucosal and sub mucosal tissues. Severe hemorrhage may result and mortality in an unprotected poultry flock may be extensive. The tissue damage can also expose the bird to bacterial infections, like *Clostridium* and *Salmonella*. The occurrence of clinical coccidiosis is directly related to the number of sporulated oocysts ingested by a bird at one time, the pathogenicity of the *Eimeria* species, the age of the infected chicken and the management system (Reid, 1990). *E. tenella* causes moderate to severe ceacal lesions. The birds become depressed, have ruffled feathers, the wings droop, have diarrhea and tend to huddle. Food and water consumption usually decreases and may become

emaciated and dehydrated. Coccidiosis is likely to occur under conditions of high stocking density which favour the build-up of pathogenic populations of the parasite. Thus, coccidiosis is especially important in intensive poultry operations where large numbers of animals are crowded together in overstocked, warm, moist, unchanging conditions. Apart from causing disease, subclinical infections cause impaired feed conversion. Since feed costs comprise some 70% of the cost of producing broiler chickens, the economic impact of coccidiosis is considerable.

Chickens are susceptible to at least 11 species of *Eimeria*. The most common species are *Eimeria tenella* (which causes the caecal or bloody type of coccidiosis), *E. acervulina* and *E. maxima*, which cause chronic intestinal coccidiosis. This parasitic infection occurs in the epithelial cells of the intestine, despite the advances in nutrition, chemotherapy, management and genetics (Magner, 1991). Most *Eimeria* species affect birds between 3 and 18 weeks of age and can cause high mortality in young chicks (McDougald, & Mattiello, 1997). In general, the losses caused by coccidiosis without including the sub clinical coccidiosis are estimated to be 2 billion USD throughout the world (O'Lorcain *et al.,* 1996).

Natural resistance to coccidiosis can be considered as a quantitative trait which is controlled by multiple gene actions. Unlike other quantitative traits, such as growth and egg production disease resistance is usually difficult to measure directly. Instead, the most commonly measured parameters for the evaluation of natural resistance are body weight gain, lesion score, feed conversion and oocyst shedding after chickens are inoculated with an equal amount of oocysts to reflect the resistance or susceptibility status (Conway *et al.,* 1993; Pinard- Van Der Laan *et al.*, 1998; Zhu *et al*., 2000). Preliminary studies on the prevalence of coccidiosis done in the past have shown that both clinical and sub clinical coccidiosis have been occurring with low prevalence rate in the local strain chicken kept under the backyard production system than in the commercially oriented production systems (Guale, 1990; Ashenafi, *et al.,* 2004). It is logical to speculate that management difference could alter the prevalence rate. However, the natural resistance of the local strain chicken to coccidiosis should be further studied to know whether the low prevalence was due to only management system or the potential natural resistance of these strains to coccidial infections.

1.2. Coccidian life cycle in poultry

The life cycle of an *Eimeria* consists of two stages:

- An exogenous stage; sporogony in the external environment
- An endogenous stage in the digestive tract of the chicken which consists of three essential phases: excystation or active departure of the sporozoites from the sporocysts, followed by several schizogonies or merogonies and gamogony.

1.2.1. Exogenous stage

Infected birds excrete oocysts with their droppings in the external environment. The oocysts excreted in this way have to sporulate in order to become infectious. Sporulation or sporogony is therefore an important stage in the parasitic cycle. It takes place outside the host in the external environment. About 48 hours at 25-28 ° C or longer if the temperature is low, are needed for the sporont inside the oocyst to transform itself into four sporocysts each containing two sporozoites (the infecting stages). Litter offers conditions of temperature, moisture and oxygenation which permit this sporulation.

Table 1. Details of Sporulation time of various *Eimeria* species

Species	Minimum sporulation time
E. acervulina	17 hrs
E. brunetti	18 hrs
E. maxima	30 hrs
E. mitis	15 hrs
E. necatrix	18 hrs
E. praecox	12 hrs
E. tenella	18 hrs

(Diseases of Poultry 11th ed. Y. M. Saif)

1.2.2. Endogenous stage

1.2.2.1. Ingestion of sporulated oocysts and excystation

The chicken becomes infected by ingesting sporulated oocysts present in the environment: litter, feed and water contaminated by faeces of oocyst-excreting chickens. These oocysts are ground mechanically in the gizzard; their shells are destroyed, releasing the sporocysts. In the duodenum, the external wall of the

sporocyst is dissolved by the action of host trypsin and bile salts and the sporozoites (infectious stages) actively come out of the sporocyst: this is called excystation.

1.2.2.2. Merogony or asexual multiplication

Depending on the species of parasite, the *mobile* sporozoites penetrate the intestinal or caecal epithelial cells. Cell invasion has not yet been completely elucidated, in particular the path adopted by the sporozoites of *E. tenella* to reach the caeca. Once the sporozoite has entered the cell, it becomes a trophozoite, the nucleus of which divides, resulting in the formation of schizonts or meronts. The meronts may contain several hundred merozoites. Once mature, the meronts cause the cells to burst and release the merozoites into the intestinal lumen. These merozoites in their turn penetrate neighbouring epithelial cells and repeat this process of asexual multiplication. Thus, depending on the species, two to four successive generations of merogonies may take place.

1.2.2.3. Gamogony or sexual reproduction

At the end of the asexual multiplications, the 2nd, 3rd or 4th generation merozoites invade the cells and develop into microgamonts (male gametocytes) and macrogamonts (female gametocytes) in accordance with an unknown determiner. The macrogamonts grows to form a macrogamete. The microgamont produces a large number of mobile microgametes with two flagella which will fertilize the macrogametes. After fertilization, the zygotes are surrounded by a thick wall ("wall-forming bodies") and are transformed into oocysts. These oocysts are eliminated from the intestinal lumen and discharged with droppings into the external environment. Thus, after ingestion of one oocyst, several days later thousands of oocysts are excreted into the environment. This period which has elapsed in the host between ingestion of an oocyst and excretion of the first oocysts in droppings is called the prepatent period. It is species-specific and varies from four to seven days according to the species of *Eimeria* in birds.

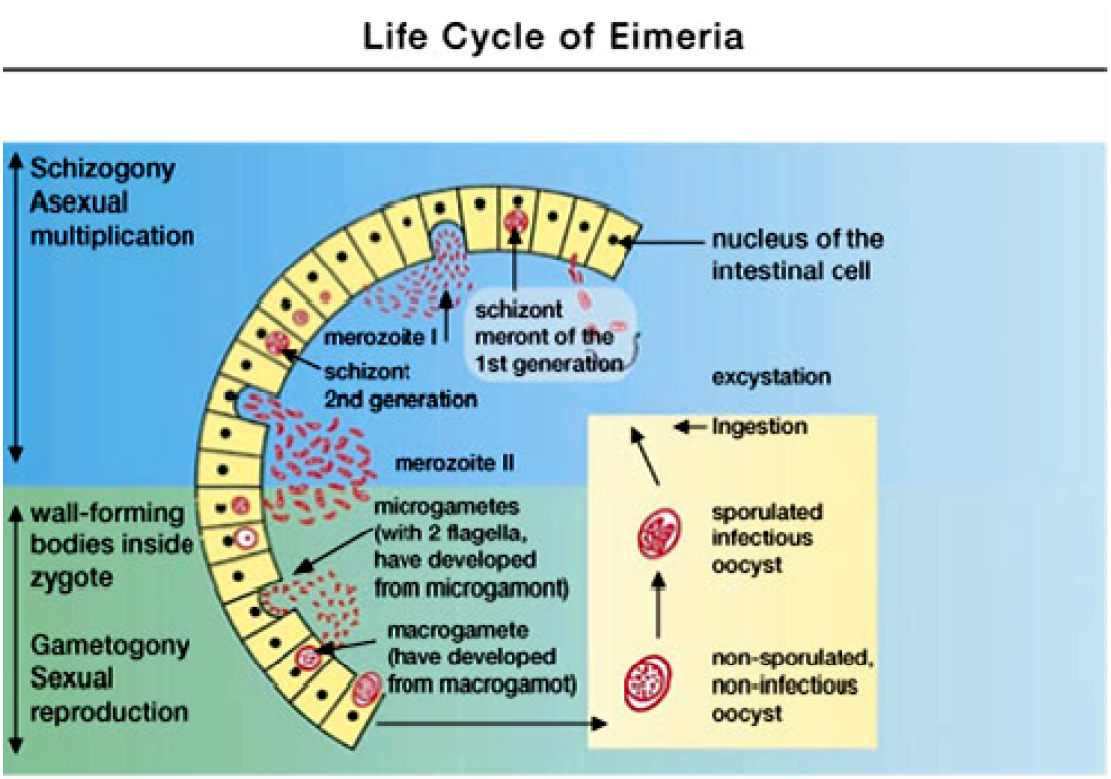

Life Cycle of Eimeria
Schizogony
Asexual
multiplication
nucleus of the
intestinal cell
schizont
meront of the
1st generation
merozoite I
schizont
2nd generation
excystation
Ingestion
merozoite II
wall-forming
bodies inside
zygote
microgametes
(with 2 flagella,
have developed
from microgamont)
sporulated
infectious
oocyst
Gametogony
Sexual
reproduction
macrogamete
(have developed
from macrogamot)
non-sporulated,
non-infectious
oocyst

Fig. 1

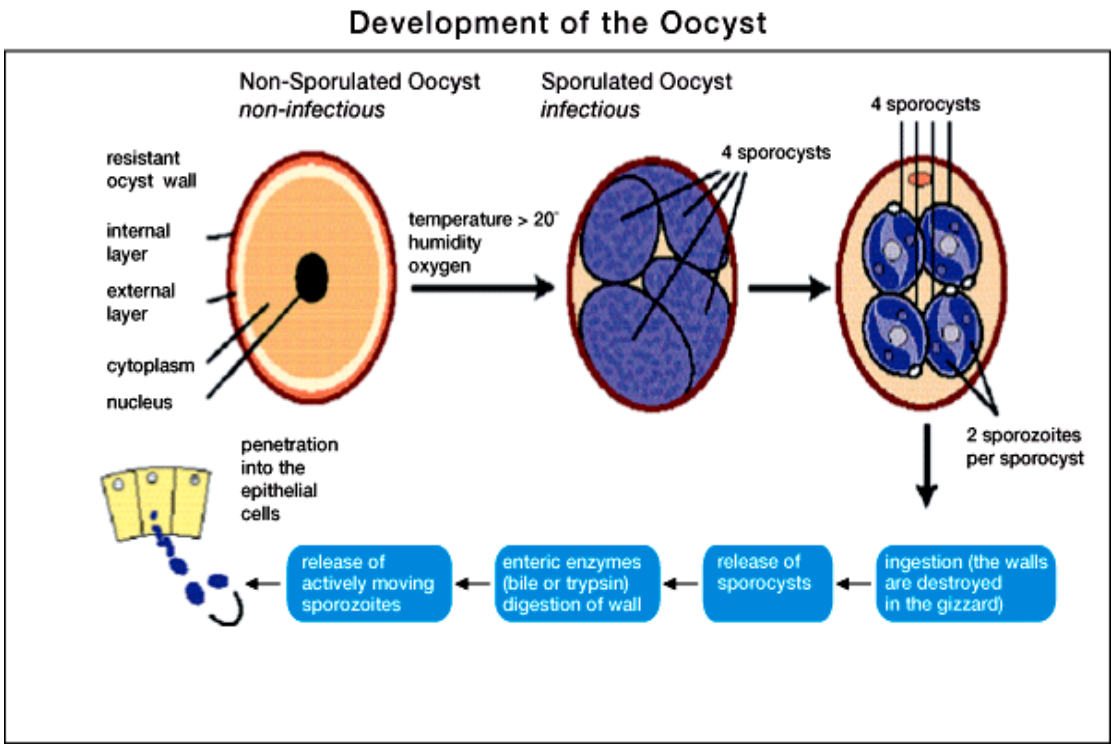

Development of the Oocyst
Non-Sporulated Oocyst
non-infectious
Sporulated Oocyst
infectious
4 sporocysts
4 sporocysts
resistant
ocyst wall
internal
layer
external
layer
cytoplasm
nucleus
temperature > 20°
humidity
oxygen
2 sporozoites
per sporocyst
penetration
into the
epithelial
cells
release of
actively moving
sporozoites
enteric enzymes
(bile or trypsin)
digestion of wall
release of
sporocysts
ingestion (the walls
are destroyed
in the gizzard)

Fig. 2

1.3. Epidemiology

The source of the infection varies and depends on the technology in the poultry industry. In extensive poultry farming the source of infection is one bird and in intensive production the source of infection is the old bird population (Hammond and Long**,** 1973). A flock, disease spreads by direct, as well as indirect contact (Williams, 2002). Oocysts that are infectious could be distributed by equipment, dust, people, rodents, wild birds as well as insects (Dimitrijevic and Ilic, 2003). Coleoptera spp., which are usually present in the broiler population, can serve as mechanical vectors (Calnek, 1997).

Distribution and prevalence is influenced by several factors: high animal density cramped on a small space, air temperature, relative humidity, different (especially different age) categories of birds at same place, feed change, quality of feed, other factors that compromise resistance to the disease and general health status of the birds (Calnek, 1997). Onset of the disease depends on the age of the bird at the time of the first infection and number of routes of the infection, as well as on ability of the bird to develop proper specific immune response (Hofstad, 1984; Ilic *et al*., 2003).

The highest incidence of coccidiosis is during spring and fall, especially when weather is cold and humid (rain). The incidence is significantly smaller during hot and dry weather conditions (Maungyai *et al*, 1990; Calnek, 1997; Razmi and Kalideri, 2000). The intensity of the infection depends on the number of oocysts that are ingested and the immune status of the bird (Hofstad, 1984). In case of release of the chicken on to the floor that was used for the previous flock Sibalic and Cvetkovic (1996), reported the acute form of the caecal coccidiosis and mortality in eight days old chicken. Infection of young chicken cannot be avoided in intensive production systems, whatever prophylactic measures have been taken. Intensive poultry production systems, high density of totally susceptible birds and many passages of the causative agent in the new bird generation, pose almost ideal circumstances for infection to persist and spread within the flock (Jordan, 1990). Heavy load of infectious oocysts on the floor is one of the most important prerequisite conditions for infection to persist in the flock (Hofstad, 1984). Clinical disease can be prevented by continuous adding of the anticoccidials in feed. However, persistence of the sub clinical disease is always a possibility. According to some authors (Braunis, 1980;

Razmi and Kalideri, 2000), sub clinical forms of the disease depend on the size of the flock. Prevalence of the sub acute form of the disease is significantly higher in flocks with more than 40,000 birds in comparison to flocks with 10,000 birds Voeten (1987) showed that sub clinical coccidiosis is most prominent from four to six week old chicken if anticoccidials are not added to the feed.

1.4. Pathogenesis

The presence of coccidia does not necessarily signify coccidiosis. The disease is more complex than a simple association of the coccidia with its host. Pathogenicity depends on the species of parasite and on the quantity of oocysts ingested, the sensitivity of the host, and the environmental situation. The different phases of parasitic development are accompanied by the rupture of the intestinal cells and effects on the physiology of the host. Pathogenicity varies in accordance with the species of *Eimeria*. It depends on the site of development, size of the different parasitic stages and their location (intestinal villi or more deeply in the crypts or cells of the lamina propria). Generally, the small stages of *E. acervulina*, schizonts of *E. maxima* are confined to the epithelial cells on the surface of the villi causing little damage since the cells at the peak of the villi are ageing (close to natural dispatch). On the other hand, the meronts of *E. tenella, E. necatrix and E. brunetti* develop more deeply in the lamina propria (deep in the intestinal wall). They become enormous and cause the rupture of blood vessels, resulting in bleeding into the intestinal lumen and death of the chickens with severe infections. These three species are highly pathogenic. The gamonts of *E. maxima*, which are very large in hypertrophied cells, often take a sub-epithelial position and are responsible for malabsorption of nutrients. *E. acervulina* and *E. mitis* are somewhat less pathogenic but often cause subclinical coccidiosis accompanied by less nutrient absorption, while *E. praecox* does not cause lesions. The pathogenic effect and the number of oocysts excreted increase in accordance with the dose of oocysts ingested. A dose of 5×10^6 oocysts of *E. acervulina* invariably produces intestinal lesions accompanied by anorexia and weight loss; however, even during very severe infections, deaths are rarely observed. On the other hand, a dose of 5×10^4 oocysts of *E. tenella* or *E. necatrix* is sufficient to kill most susceptible chickens. Under natural conditions, chickens are infected by several species, and there may be competition between species colonising the same intestinal site.

1.5. Prevention

Coccidial oocysts are extremely resistant to environmental conditions and disinfectant agents, therefore, eradication of coccidiosis from chicken houses by litter removal, cleaning and disinfection is not possible. Anticoccidial agents added to the feed have been used since the mid 50s and have been instrumental in allowing the expansion of the broiler industry to what it is today. The effective use of anticoccidial drugs over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. Numerous anticoccidial drugs were introduced, many of which are available and used today. However, there is increasing concern about rising levels of drug resistance (Chapman, 1997).

Today, the poultry industry faces many challenges; among them is the controversy over the use of growth promoting antibiotics. The use of antibiotics as a feed additive is a common practice in livestock production. Low levels of antibiotics are used in poultry diets mainly to reduce disease incidences but they also lead to an increase in growth performance and feed efficiency. The addition of sub-therapeutic levels of antibiotics to poultry diets is beneficial; unfortunately, consumer perceptions are that edible poultry tissues are contaminated with harmful concentrations of drug residues. Consumers and some scientists believe that there may be a link between the agricultural use of antibiotics and antibiotic resistance in humans and regulatory agencies such as the United States Food and Drug Administration, the Centers for Disease Control and Prevention, and the World Health Organization all agree that sub therapeutic use of antimicrobials in animal feed should be restricted.

Many anticoccidial drugs have also been introduced in the poultry industry all over the world. Various anticoccidial feed additives, predominantly polyether ionophorous antibiotics, have been developed and used. While effective for avian coccidiosis, the continuous use of these anticoccidial drugs have led to the emergence of drug-resistant strains. Furthermore, drug residue in the poultry products is also un-desirable for the consumer. Therefore, there is need to find out the safe alternatives for the control of avian coccidiosis. In this context, a number of plants and herbal products have been found to be effective for a broad range of parasites such as protozoa, arthropods and helminths. Mushrooms also have high medicinal value and

thus can be screened for the presence of anticoccidial substances. In Asia and some tropical countries of Africa, edible and medicinal mushrooms such as *Pleurotus ostreatus* and *Ganoderma lucidum* are used as food supplements and medicines to improve various parameters of human health and immune functions in certain disease conditions. A lot of literature is available on the beneficial effects of mushrooms, particularly the *G. lucidum*, which is cultivated in China and Japan, and other western nations. The wild species of *G. lucidum* also grows in abundance in Kashmir on wood and tree stumps during the rainy season. Mushrooms, like probiotics are natural ingredients that contain bioactive chemical substances, or polysaccharides, proteins, crude fibres, unsaturated fat, minerals, vitamins, essential amino acids and organic acids that can be used as good sources of food supplements and medicines to promote health and production. The possibility of a ban on the use of antibiotics in poultry feeds demands the need for studies to evaluate alternatives. In the light of above mentioned facts the study becomes important as it can provide a cheap alternative for the control of coccidiosis. Also the problem of health hazards due to drug residues in meat can be overcome. Further the use of medicinal mushroom extracts can bless us with a drug with better efficacy for controlling coccidiosis.

1.6. Mushrooms as potential source of drugs

Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand (Chang and Miles, 1992). Mushrooms belong to only two subdivisions of fungi; the vast majority is basidiomycetes, and a few are ascomycetes. Worldwide mushroom species are as high as 10,000 with about 700 edible, 50-200 medicinal, and 50 poisonous species. It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity (Anke 1989). Dioscorides, first century Greek physician, knew that Laricifomes (*Fomitopsis officinalis)* (Fomitopsidaceae) can be used for treatment of “consumption”, a disease now known as tuberculosis (Stamets, 2002). Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world’s greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl,

1983). Due to high amount of proteins, they can be used to bridge the protein malnutrition gap.

Edible mushrooms have been widely utilized as human foods for centuries and have been appreciated for texture and flavor as well as some medicinal and tonic attributes (Manzi *et al*., 2001). However, the awareness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (Cheung *et al*., 2003). Mushrooms are considered as healthy food because they are low in calories and fat but rich in proteins and dietary fibers (Manzi *et al*., 1999). Mushroom protein contains all the nine essential amino acids required by humans. In addition to their good protein content, mushrooms are a relatively good source of the nutrients like phosphorus, iron and vitamins, including thiamine, riboflavin, ascorbic acid, ergo sterol, and niacin (Barros *et al*., 2008).

Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times, especially in Japan and China. A number of medicinal mushrooms, such as *Aleurodiscus, Coprinus, Pleurotus, Polyporus, Poria, Psathyrella,* and *Tricholoma* spp. are rich sources of β-glucan, proteoglucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarins, alkaloids etc and also have been used extensively in traditional medicine for curing various types of diseases. Besides mushrooms have been known to possess antimicrobial, antiviral, anticancerous, antiinflamatory, immunomodulating and other central activities (Benedict and Brady, 1972; Wang *et al.*, 1996; Jose *et al*., 2002; Wasser, 2002; Wang *et al.*, 2002). The effects of different mushroom extracts on pathogens and microorganisms have been studied by a very large number of researchers in different parts of the world (Jonathan and Fasidi, 2003; Rosa *et al*., 2003; Gezer *et al*., 2006; Turkoglu *et al*., 2006; Barros *et al*., 2007; Gbolagade *et al.*, 2007; Turkoglu *et al*., 2007; Akyuz and Kirbag, 2009; Mwita *et al.*, 2010; Sivaprakasam *et al.*, 2011).

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. It is therefore not surprising that antimicrobial compounds with more or less strong activities could be isolated from many mushroom species and some proved to be beneficial for humans (Lindequist *et al.*, 2005). In early studies

diverse antibiotic activity was detected in basidiocarp or mycelia culture extracts of more than 2000 fungal species (Rosa *et al*., 2003). Antimicrobial activities of basidiomycete strains from different countries were screened in submerged culture (Rosa *et al.,* 2003; Wasser *et al.,* 1999; Ezeronye *et al.,* 2005). Similarly, Rosa *et al.* (2003) detected 14 mushroom isolates with significant activity against one or more of the target microorganisms. Zjawiony (2004) observed that 75% of polypore fungi that have been tested show strong antimicrobial activity. In this context, screening of medicinal mushrooms can bless us with novel drugs for controlling coccidiosis.

Chapter: 2

Review of Literature

On reviewing the available literature, it was found that wide range of medicinal mushrooms have been used to treat parasitic infections in man and animals. Medical usage of mushrooms with regard to antimicrobial and antitumor activity is very diverse as evident from the review of literature. Keeping all this in view, the present work entitled "Comparative efficacy of extracts of some medicinal mushrooms against coccidiosis in broilers**"** was designed to screen some medicinal mushrooms found in Kashmir valley for their anticoccidial activity.

Laczay *et al*. (1995) studied the therapeutic efficacy of sulphachlorpyrazin and toltrazuril in broilers experimentally infected with *E. tenella* both in battery and floor pen systems. The results of battery studies revealed that, both drugs prevented coccidiosis related mortality and reduced weight gain to a similar degree, but toltrazuril was more effective in reducing cecal lesions and faecal scores when medication were started 24 hr P.I. On the other hand, when medication was delayed to 72 hr P.I., Sulphachlorpyrazin was effective in preventing reduction of weight gain and cecal lesions of *E. tenella***.** Under simulated conditions, both the drugs had the same efficacy without major differences between them.

Mizu *et al*. (1998) evaluated the broiler performance in terms of weight gain, feed consumption, feed efficiency and production number. Litter dampness was

determined and coccidial oocyst populations were counted at different weeks of age. The depth of litter didn't significantly affect live weight gain, feed consumption, feed conversion ratio, livability or production number. Variation in moisture contents of litter was observed but the coccidial oocysts count per gram of litter was within safety level and therefore, they conclude that there was no outbreak of coccidiosis in any group. They also suggested that rice husk can be used as litter at depths of between 20 and 50 mm during winter to raise broilers without affecting performance characteristics and health of birds.

Hirasawa *et al.* (1999) extracted three kinds of substances by using chloroform, ethylacetate or water as solvents from dried Shiitake mushrooms (*Lentinus edodes*).These substances have been reported to possess efficient antibacterial activities against *Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Prebotella* spp., and *Porphyromonas* spp. of oral origin. Their result showed that chloroform extracts possess bactericidal activity against growing and resting bacterial cells of *S. mutans* and *P. intermedia*, whereas the other two extracts showed bacteriostatic activity against both growing and resting bacterial cells of *S. mutans* and resting bacterial cell of *P. intermedia*.

Hooge *et al.* (2000) studied the beneficial interaction between ionophores (salinomycin or monensin) and bicarbonates (sodium and potassium bicarbonate) lead to lowering the mortality of chicks. They observed that this combination improved body weight, FCR and mortalities. They also reported that Salinomycin improved coccidial lesion score, body weight, FCR and mortality compared to monensin.

Soomro *et al.* (2001) observed the clinical, gross and histopathological changes in poultry birds suffering from coccidiosis. The symptoms observed were loss of appetite, unthriftiness, greenish or reddish diarrhea and affected birds were showing their comb and wattles pale and anemic. Their postmortem examination revealed intestine extremely balooned, having petechial haemorrhages, oedematous, necrosis and sloughing of intestinal and caecal epithelium. They also performed histological examination, which showed leakage of blood, oedema, necrosis, disruption and loss of villi.

Ishikawa *et al.* (2001) evaluated the antibacterial activity of 35 isolates of *Lentinula edodes*, a shiitake mushroom against *Bacillus subtilis* by diffusion

technique in agar with a semi-solid overlay. Their result showed that all isolates inhibited *B. subtilis* and the isolate Le1 promoted the formation of the largest inhibition zone. They also reported that *L. edode* Le1 also presented antibacterial activity against foodborne pathogens and food contaminant bacteria, particularly Grampositive species.

Lam *et al*. (2001) isolated a peptide with a molecular weight of 8 kDa and N-terminal sequence closely resembling that of ubiquitin from fruiting bodies of the mosaic puffball mushroom *Calvatia caelata.* The peptide is known to inhibit the translation in the cell-free rabbit reticulocyte lysate system and exhibited *N*-glycosidase activity. It potently inhibited proliferation of spleen cells with an IC50 of about 100 nM as indicated by the suppression of [*methyl*- 3H] thymidine uptake. Their result depicted that the viability of breast cancer cells which was reduced to half at a ubiquitin concentration of about 100 nM .

Jordan and Zjawiony (2003) studied the biological activity of natural products isolated from aphyllophorales, many of which are known as polypores. Their results showed that 75% of polypore fungi have been reported to posses antimicrobial activity and thus constitute a good source for developing new antibiotics. They also reported numerous compounds from these fungi which display antiviral, cytotoxic, and/or antineoplastic activities.

Daba *et al*. (2003) demonstrated the anti-cancer effect of polysaccharides isolated from higher basidiomycete mushrooms using different cancer cell lines. These polysaccharide extracts showed potent antitumor activity against sarcoma 180, mammary adenocarcinoma 755, leukemia L-1210 and a host of other tumors. The antitumor activity was mainly due to indirect host mediated immunotherapeutic effect. These studies are still in progress in many laboratories and the role of the polysaccharides as immunopotentiators is especially under intense debate.

Parsani *et al*. (2003) investigated the prevalence of gastrointestinal parasites at Kamla Nehru zoological garden Gujrat and out of 138 group faecal samples, 85.48% samples were found positive for *Eimeria* species. It was concluded that coccidian and nematode infection was higher in those birds compared to other infections because coccidia and nematodes are having direct life cycle patterns which helps the parasite to remain in the cages and reinfect the birds.

Etuk *et al*. (2004) worked on-farm prevalence and management of poultry coccidiosis, which showed that in the previous 12 months, 3,327 (29.36%) birds out of 11,333 encountered in the 30 farms suffered from coccidiosis and the overall mortality rate was 2.63%. They recorded the highest prevalence rates in the rainy season (12.7%), among birds managed in deep litter (26.69%), birds 1-5 weeks old (18.75%), layers (22.29%) and Harco strain (26.42%). In this study sixty percent of the farms consulted veterinarians for diagnosis and treatment especially at first incidence while 34.94% indulged in self-diagnosis. Good sanitary and hygiene practices were being employed in 50% of the farms as the major preventive measure. Combined administration of anticoccidial drugs and removal of litter (43.33%) ranked highest as control measure.

Guo *et al.* (2004) investigated the effects of polysaccharide extracts from two mushrooms, *Lentinus edodes* (LenE) and *Tremella fuciformis* (TreE), and a herb, *Astragalus membranaceus* (AstE), on cellular and humoral immune responses of *Eimeria tenella*-infected chickens. The results suggested that supplementation with mushroom and herb extracts resulted in enhancement of both cellular and humoral immune responses in *E. tenella*-infected chickens.

Gbolagade and Fasidi (2005) investigated the antimicrobial activities of methanolic extracts of five Nigerian mushrooms – *Auricularia polytricha, Corilopsis occidentalis, Daldinia concentrica, Daedalea elegans and Tricholoma lobayensis* using filter paper disc and hole diffusion methods. The result showed that all the mushrooms were found to exhibit various degrees of antagonistic effects against the tested microorganisms indicated the clear zone of inhibition produced by the bacteria and fungi around the tested mushroom extracts.

Lindequist *et al.* (2005) described the pharmacologically active compounds isolated from mushrooms.These compounds and complex substances were reported to possess antimicrobial, antiviral, antitumor, antiallergic, immunomodulating, anti-inflammatory, anti-atherogenic, hypoglycemic, hepatoprotective and central activities.

Fanatico (2006) studied the coccidiosis in terms of its life cycle, transmission in free-range production, management in the brooder and on pasture, natural treatments, drugs, and vaccines. He observed that coccidiosis can be handled without

medication by careful management, especially during brooding, and adequate pasture rotation; however, on a larger scale, it will be difficult and vaccines could be important alternative to drugs in organic production.

Badran (2006) compared the effect of additive and nonadditive or probiotic feed supplement (*Enteroccus faecium*) in the: diet containing anticoccidia on performance of broiler, diet without anticoccidia or vaccine (control) and diet containing coccidia vaccine on performance of broilers. He observed that the probiotic (*Enterococcus faecium*) which is a common component of intestinal microbial of normal human and animals will immunize the poultry against the disease.

Biu *et al.* (2006) observed the anticoccidial efficacy of the aqueous neem leaf extract in comparison to amprolium. The results of this study showed that the aqueous extract dose of 800mg/kg compared favourably with 10mg/litre of amprolium in treating the disease, both showed 100% survival rates for infected and treated chickens with zero oocyst per gram at day 4 post treatment. They further reported that the mean weight (grams) of infected and treated chickens improved significantly ($P<0.05$) at day 7-post treatment

Kitandu and Juranova (2006) studied that anticoccidial drugs for controlling of chicken coccidiosis in broiler industry which has played a major role in the growth of this industry. They also suggested the use of live vaccines in controlling of the disease.

Khan *et al.* (2006) determined the prevalence of eimeriosis in poultry and identified the potential risk factors for its spread in Rawalpindi/Islamabad area of Pakistan. Of 359 gut samples (suspected for harbouring eimeriosis) examined, 258 (71.86%) were found infected. Four species of *Eimeria* (*E. maxima*, 34.10%, *E. tenella*, 30.62%, *E. mitis*, 13.95% and *E. necatrix*, 7.75%) were recorded. They reported that the prevalence of Eimeriosis was highest in the month of September (89.74%), while lowest during June (28.57%) and found that the disease was more common at the farms where the litter was wet and not managed properly.

Barros *et al.* (2007) evaluvated the antimicrobial activity of the wild mushrooms, *Lactarius deliciosus* and *Lactarius piperatus*, against Gram positive and Gram negative bacteria and fungi, and they correlated this to amounts of phenols,

flavonoids, ascorbic acid, _-carotene, and lycopene present in the immature and mature fruiting bodies

Carvalho *et al.* (2007) evaluated the two species of basidiomycetes, *Lentinula boryana* and *Lentinula edodes*, for their antibacterial activities, biomass production and growth in different culture media. They observed that *L. boryana* showed the largest biomass production in both culture media, while as *L. edodes*, presented significant differences in growth in different culture media. Their results showed that both basidiomycetes *L. boryana* and *L. edodes* possess antibacterial activity against *B. cereus* and *S. aureus*, although only *L. edodes* was active against *S. mutans.*

De Franceschi, *et al*. (2008) analysed systematically data on broiler chickens for the period of ten years. They reported 80 % of the samples were positive to coccidiosis and 58.5% were sub-clinical, the 34% clinical degree 1 and the 7.5% clinical degree 2 and 3.

Khan *et al.* (2008) studied the efficacy of some herbal and homeopathic preparations against coccidiosis on the basis of weight gain, feed conversion ratio, oocyst count and mortality rate. They treated chicks with *Polygonum bistorta* Linn. (Anjbar), *Agele marmelos* (Bael), Merc sol. (*Mercurius solubilis*) and Darvisul liquid and found that, *A. marmelos* (Bael fruit) and Darvisul liquid showed better results in terms of weight gain, feed consumption, oocyst count as compared with *P. bistorta* Linn. (Anjbar) and Merc solution.

Ogbe *et al.* (2008) evaluated the immune enhancing effect of a wild *Ganoderma* mushroom (*Ganoderma lucidum*) to infectious bursal disease vaccine. Their results in both qualitative and quantitative Agar gel precipitation test, showed positive response in all the vaccinated groups at 6 weeks of age and Enzyme-Linked Immunosorbent Assay revealed seroconversion at 4 weeks of age in the vaccinated birds. Their study highlighted the benefits of wild *Ganoderma lucidum* in enhancing immune response of chickens to infectious bursal disease vaccination.

Vega *et al*. (2008) carried out a research programme which aimes at using fungal endophytes-mediated plant defense as a novel biological control mechanism against the coffee berry borer, the most devastating pest of coffee throughout the

world. This paper reviews the possible mode of action of entomopathogenic fungal endophytes.

Ogbe *et al.* (2009) conducted an experiment to study the effect of aqueous extract of a wild mushroom *Ganoderma lucidum* in coccidian-infected broilers on the basis of changes in weight gain, faecal oocyst count and packed cell volume. Their results showed that the infection of broilers with *Eimeria tenella* causes bloody diarrhoea as a result of damage to the intestinal mucosa leading to depression of feed intake and loss of body weight. They also confirmed that treatments with *Ganoderma lucidum* resulted in amelioration of clinical signs of bloody diarrhoea and reduction of faecal oocyst count and improved feed intake and weight gain. The treatments did not adversely affect PCV in *E. tenella*-infected and un-infected broilers.

Oyetayo (2009) investigate the antioxidant and antimicrobial potentials of extracts obtained from four wild mushrooms, *Termitomyces clypeatus* (TCE), *Termitomyces robustus* (TRE), *Lentinus subnudus* (LSE) and *Lenzites* species (LZE) collected in Nigeria. They documented that extracts were able to inhibit the growth of all indicator organisms and also observed that LSE and LZE, showed better antimicrobial effect. Their results suggested that extracts obtained from the four wild mushrooms may serve as sources of new bioactive compounds with effective antioxidant and antimicrobial activity.

Vinayaka *et al.* (2009) investigated proximate composition, antioxidant, anthelmintic and insecticidal efficacy of methanolic extract of a macrolichen *Ramalina conduplicans* (Ramalinaceae). The results showed that methanolic extract exhibited marked antioxidant activity. They also studied the antihelmintic activity of methanolic extract against adult Indian earthworms, and reported a dose-dependent inhibition of spontaneous motility.

Akyuz *et al*. (2010) investigated the antimicrobial activity of methonolic extract of *Pleurotus* spp., *T. boudieri* and *Agaricus. bisporus* according to the disk diffusion method by using *Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Candida albicans, C. glabrata, Trichophyton* spp. and *Epidermophyton* spp. At the end of the experimental studies, the methonolic extracts of *Pleurotus spp., T. boudieri* and *A. bisporus* were shown to inhibit the growth of microorganisms (7.5-15.5 mm).

Allen and Jenkins (2010) observed the gross pathology of chickens when challenged with different doses of sporulated *Eimeria praecox* oocysts. There was moderate but significant weight gain reduction at all infective doses. Substantial reduction in plasma carotenoids and moderate but significant increases in plasma NO_2^- + NO_3^- were observed only at the higher doses when measured at day 6 post challenge (PC). Daily monitoring of chickens after challenge with 5×10^4 oocysts revealed an inflammatory response in the duodenum and jejunum beginning at day 1 PC that was associated with a significant increase in levels of plasma NO_2^- +NO_3^- , which peaked at day 4 PC. A moderate, uniform hyperplasia of the small intestine and significant depression of plasma carotenoids were observed on days 4–6 PC. Plasma NO_2^- +NO_3^- decreased to control levels by day 6 PC. All infections were accompanied by production of a mucoid exudate in the duodenum and jejunum, which became thick and opaque by 4 days PC. These observations indicate that the acute host response to primary infection with *E. praecox* is both different from experimental infections with other *Eimeria* spp., such as *Eimeria acervulina, Eimeria maxima*, or *Eimeria tenella*.

Bera *et al*. (2010) evaluated the economic loss to poultry industry considering the major economic parameters. The estimation has revealed that commercial broiler industry is a major sufferer due to coccidiosis wherein 95.61 per cent of the total economic loss occurs due to the disease. The commercial layer industry shares 3.53 per cent economic loss, mainly due to cost of chemoprophylaxis and reduced egg production. A comparison across economic traits has revealed that loss is maximum due to reduced body weight gain, followed by increased FCR (23.74%) and chemoprophylaxis (2.83%) in the total loss due to coccidiosis in broiler industry of India. The overall comparison of economic traits for all the types of poultry sector has shown that reduced body wt gain and increased FCR are the major parameters from which 68.08 per cent and 22.70 per cent annual loss has occurred in the total loss from coccidiosis in India during the year 2003-04. The total loss due to coccidiosis has been found to be of Rs 1.14 billion (approx) for the year 2003-04.

Jonathan and Awotona (2010) studied the invitro antagonistic effect of the ethanol, methanol and distilled water extracts of the fruit bodies of three *Ganoderma* species namely *G. lucidium, G. applanatum and G. australe* against some disease

causing microorganisms. Both crude and pure extracts of these *Ganoderma* species exhibited various degree of inhibition of growth against the test organisms.

Kumar *et al*. (2010) studied the antibacterial, anthelmintic and antioxidant activity of a macrolichen *Parmotrema pseudotinctorum* collected from forest area of Bhadra wildlife sanctuary. The extract exhibited marked antibacterial activity. The minimum inhibitory concentration of the extract was found to be lesser in case of Gram negative bacteria than Gram positive bacteria. The lichen extract exhibited a dose dependent inhibition of spontaneous motility.

Mwita *et al*. (2010) studied the antimicrobial activity of crude ethyl acetate extracts of *Coprinus cinereus* using the agar well method. The results showed the antimicrobial activity only in capping and post capping stages of the mushrooms and the activity generally increased with increased percentage of manure supplementation. These findings have shown that Tanzanian edible *C. cinereus* mushroom contains antimicrobial compounds and chicken manure could be used in the cultivation of the mushroom to increase the production of active secondary metabolites, which could be used as lead compounds for discovery of new and more effective drugs against microbial infections.

Manjunathan *et al*. (2010) investigated the in vitro antimicrobial properties of *Lentinus tuberregium* using four different solvent systems (Hexane, Dichloromethane, Chloroform and Ethyl acetate). The activity was evaluated by well diffusion tests using bacteria and yeasts. Vancomycine and fluconazole were used as positive controls for bacteria and yeasts, respectively. The crude extracts of *Lentinus tuberregium* have relatively high antimicrobial activity. Among the four organic extracts ethyl acetate extract was found to be showed more effective and inhibited the growth of human pathogenic bacteria and yeast.

Naphade *et al*. (2010) studied the efficacy of homoeopathic medicine (*Mercurius Corrosivus*) against experimental caecal coccidiosis in broiler chicks. The infection was raised by giving the dose of 50,000 sporulated oocyst of *E. tenella*. Gross pathological changes observed in an infected untreated control group were paleness of the mucosal membrane, ballooning of the caeca with clotted and non clotted blood. However, less severe changes were observed in the groups treated with Amprolium and *Mercurius Corrosivus*. They also studied on the histopathology of

intestinal caeca in different groups of birds (treated and untreated). In the infected untreated control group the intestinal mucosa was hypertrophied containing schizonts and gametocytic stages of coccidial pathogen. There was desquamation of intestinal mucosa and denudation of intestinal villi cells, where as the other groups showed less severe histopathological changes. They finally concluded that the drug was found to be effective as a curative remedy against experimental caecal coccidiosis.

Ogbe *et al.* (2010) evaluated the haematological parameters and the histopathological lesions in broilers treated with aqueous extract of wild *Ganoderma* sp. The results showed the values of haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) were within normal range in all the groups and seemed to bear no direct relationship to the treatment using either the wild mushroom or amprolium. Histopathology showed mild lymphocytic infiltration in the liver of the broilers. The lesions could not be linked to the use of mushroom or amprolium, as both treated and untreated birds had similar lesions in their organs.

Lee *et al.* (2010) examined the effects of organic extracts from milk thistle (*Silybum marianum*), turmeric (*Curcuma longa*), reishi mushroom (*Ganoderma lucidum*), and shiitake mushroom (*Lentinus edodes*) on innate immunity and tumor cell viability. In vitro culture of chicken spleen lymphocytes with extracts of milk thistle, turmeric, and shiitake and reishi mushrooms induced significantly higher cell proliferation compared with the untreated control cells. They further investigated stimulation of macrophages with extracts of milk thistle and shiitake and reishi mushrooms, but not turmeric, resulted in robust nitric oxide production to levels that were similar with those induced by recombinant chicken interferon-γ. All extracts uniformly inhibited the growth of chicken tumour cells in vitro at the concentration of 6.3 through 100 mg/ml.

Quereshi *et al.* (2010) evalvated the antimicrobial activity of various extracts (40□g/ml) of *Ganoderma lucidum* against six species of bacteria*: Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Salmonella typhi* and *Pseudomonas aeruginosa*. These results have shown that acetone extract exhibited maximum antibacterial activity (31.60±0.10), while the most susceptible bacterium observed was *Klebsiella pneumoniae.*

Ch *et al*. (2011) investigated the antimicrobial activity of 90% ethyl acetate and antioxidant activity of fruiting bodies of four edible mushrooms against different bacteria using agar well diffusion method with ampicillin as positive standard reference to determine the sensivity of the bacterial strains. The result showed that the two mushrooms were having significant inhibitory activity against *Staphylococcus aureus* which is a normal inhabitant in humans and the antioxidant activity.

Ezeontyejiakui *et al*. (2011) investigated the effect of inclusion of mushroom (*Pleurotus cystidiosus)* to substitute lysine in the diet of broiler chicks on the basis of mean weight gain and mean feed intake. Twenty four broiler chicks were subjected to two different dietary treatments (Diet I contained 0.22% of mushroom while Diet II contained 0.22% of synthetic Lysine and was used as control). Student t- test showed that there was no significant difference (P>0.05) in the mean weight gain for the chicks on the two treatments and mean feed intake for the chicks on the two treatments. Consequently, their results showed that mushroom can be used to substitute lysine in the diet of broiler chicks.

Sivaprakasam *et al*. (2011) tested the antibacterial and antifungal activities of aqueous and methanolic extracts of *Trametes hirsuta* against five pathogenic fungi like *Penicillium* sps., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucour indicus* and five bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans* by well diffusion assay.The result showed that the maximum antibacterial activity of aqueous extract of *Trametes hirsuta* was found 33 mm at 200 mg against *Staphylococcus aureus* than that of methanol extract. The significant antifungal activity of aqueous extract was found 46 mm at 200 mg against *Aspergillus flavus* than that of methanol extract. The antimicrobial activity was showed to be concentration dependent.

Sridhar *et al.* (2011) carried out a study to evaluate the antibacterial and antifungal activity of methanol and aqueous extract of fruit bodies from *Ganoderma lucidum* (Fr.) on five bacterial pathogens such as *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans* and five fungal strains *Penicillium sps., Aspergillus Fumigatous , Aspergillus niger*, *Aspergillus flavus* and *Mucour indicus*. For antimicrobial test, well diffusion

technique was used and the zone of inhibition of microorganisms was measured in mm. The fruit body of *G. lucidum* showed potential antimicrobial activities against the selected strains an maximum inhibition zone 31mm was recorded from 200mg of aqueous extract of *G. lucidum* fruit body against *Salmonella typhi* and *Staphylococcus aureus* and minimum (10mm) by the *Escherichia coli* at 50 mg of extract. The methanolic extract showed the maximum antifungal activity 30mm inhibition zone was recorded from 200mg of extract against *Mucor indicus* and minimum 3mm by 50 mg of extract against *Aspergillus flavus*.

Tammina and Hariprasad (2011) studied the phytochemical and antimicrobial activity of common cultivated mushroom, *Agaricus bisporus*. The different fractions of methanolic extracts of the whole mushroom of *Agaricus bisporus* was subjected to preliminary phytochemical and in-vitro anti-microbial studies.The result showed that the fraction II of the methanolic extract inhibited the growth of all the test bacterial species whereas fraction III and fraction IV have shown weak antibacterial activity.

Willis *et al.* (2011) investigated Fungus Myceliated Grain (FMG) feed inclusion strategies for broilers and the effects of this feed on natural *Eimeria* oocyst excretion and bird performance. Results showed that broilers in treatments 1 and 2 produced the highest counts of *Eimeria*, which was significantly higher than that of treatment 6 ($p<0.05$) with the lowest count of *Eimeria*. Mortality was not significantly influenced by treatments. they reported that there were significant differences in the live and bursa weights, but not in the relative bursa percent. The results suggest the best response in terms of anticoccidial protection occurs with the 10% inclusion in the growers feed and for body weight at the 5% inclusion level in the starter feed.

Kavyani *et al.* (2012) studied the effects of different levels of edible mushroom (*Agaricus bisporus*) in comparison with an antibiotic growth promoter (flavophospholipol) on performance, carcass characteristics and immune responses of broiler chicks. The results indicated that supplementing broiler diet with 30 g mushroom/kg body wt. could induce favorable influences on immune responses of broilers without any adverse effects on performance criteria.

Ogbe and Affiku (2012) evaluated the effects of polyherbal aqueous extracts from *Moringa oleifera*, Gum Arabic and wild *Ganoderma lucidum* as safe and natural

alternative to reduce over-dependence on the use of antibiotic (growth promoters) on the basis of growth performance and haematological parameters of broiler chickens. the results of this study showed that *Moringa oleifera* leaves, gum arabic and wild *Ganoderma lucidum* contained appreciable amount of crude protein, dietary fibre, fatty acids and minerals, which are nutritional requirements of broiler chickens. Moreover their results also showed that the polyherbal extracts had no adverse effects on heamatological parameters of broilers. The heamatological values were all within the normal range.

Skalicka-Woźniak *et al.* (2012) studied the antibacterial activity of polysaccharides in *Ganoderma lucidum* fruit bodies *in vitro* using micro-dilution broth method. The panel of eight reference bacterial strains was used. All the polysaccharide samples tested showed the broad spectrum and the moderate antibacterial activity. *Micrococcus luteus* ATCC 10240 strain was the most sensitive with *MIC* (minimal inhibitory concentration) = 0.63 − 1.25 mg/mL.

Sonawane *et al*. (2012) studied the pharmacological activities of acetone, methanol and ethyl acetate extracts prepared from *Phellinus* and *Ganoderma.* The antimicrobial assay showed zone of inhibition against different strains of Acinetobacter and acetone extract gave best results. The extract of *Phellinus* was found to be stable in alkaline and acidic conditions.They concluded that *Phellinus* could be used as a potent herbal drug and as an oral antibiotic.

Wills *et al*. (2012) evaluate the feeding of four mushrooms separate (Shiitake, Reishi, Oyster, Cordyceps) and combined via Fungus Myceliated Grain (FMG) on broiler chicken performance after an *Eimeria* challenge at 14 days of age. Result of the study showed that the Shiitake mushroom outperformed the other mushrooms as a stand alone or in combination with regards to performance and *Eimeria* protection. Besides Cordyceps alone at the 5% inclusion level should not be fed in rations for broiler chickens due to the significant repression of body weight but instead in combination with others, or at a reduced level, due to its superior protection against *Eimeria spp.* These results suggest that dietary FMG supplementation could improve growth performance in coccidia-infected broilers possibly through enhanced immune function. This research adds to the small body of knowledge for utilizing different

mushrooms via FMG as alternatives to drug/antibiotics and hormone replacement in poultry rearing.

Hossain *et al*. (2013) evaluated the anticoccidial efficacy of oyster mushroom on the basis of oocyst count per gram (OPG) of faeces, weight gain, morbidity, mortality, necropsy findings and histopathology. Results showed that the highest performance ($p<0.01$) of OPG count, body weight gain ($p<0.01$), morbidity and mortality was detected on day 28 where oyster mushroom was supplemented @ of 100 and 150 mg/kg body weight respectively with feed from 2-28 day of age, whereas broiler chicks of group B were infected and non-supplemented thus showed the lowest performance. So it could be concluded that supplementation of 100-150 mg oyster mushroom/kg body weight reduces the development of cecal coccidiosis in chicken.

Willis *et al. (*2013) conducted an experiment to evaluate the feeding of four medicinal mushrooms: Shiitake (*Lentinus edodes*), Reishi (*Ganoderma lucidum*), Oyster (*Pleurotus ostreatus*) and Cordyceps (*Cordycepss inensis*) on performance, blood Parameters and natural coccidiosis infection in floor-reared broilers. The results from this study indicate that different fungi and levels of their inclusion into the basal feed can impact production performance responses significantly and enhance the overall health of broiler chickens. The study also reveals that there are ample advantages to using natural medicinal mushrooms as immunonutrition verses antibiotics to enhance health and production performance of broiler chickens.

Chapter: 3

Material & Methods

Methodology involves a series of aspects from field trip collections to the observations including selection and collection of the medicinal mushrooms, preparation of their extracts, usage of instruments and formation of protocols. All this requires good build of mind and soft technical hand to handle the material and procedure in a true scientific manner.

3.1. Evaluation of anticoccidial activity of medicinal mushrooms

3.1.1. Collection of mushrooms

The mushrooms to be evaluated were selected according to literature and their usage in parasitic diseases. In the present study two medicinal mushrooms namely *Ganoderma applanatum and Fomes fomentarius* were evaluated for their in vivo anticoccidial activity against coccidiosis in broilers. The mushrooms were identified and authenticated from Mycology section Department of Botany, University of Kashmir, Srinagar, India. Voucher specimens (Table 2) were deposited in KASH (Kashmir University Herbarium).

Table 2. Details of mushrooms collected

Mushroom species	**Family**	**Parts used**	**KASH NO.**
Ganoderma applanatum	Ganodermataceae	Fruiting body	1801
Fomes fomentarius	Polyporaceae	Fruiting body	1802

3.1.2. *Ganoderma applanatum* (Ganodermataceae)

Ganoderma applanatum known as conk is a general term used for a fungus that destroys wood and digest the brown lignin as food source and leave behind the white cellulose. *G. applanatum*, appropriately dubbed a shelf fungus due to its shape, a fan-

shaped polypore ranges from 30-70 cm long, that makes it noticeable in the woods. It has a thick, hard, lumpy, brown top with several radiating zones. The spore surface is ochre in color and after scratching the spore surface becomes brown. The pores of the spore surface are tiny and regular in shape. *G. applanatum* creates a new pore surface each year, giving it a "stacked" appearance

3.1.3. *Fomes fomentarius* (Polyporaceae)

Fomes fomentarius (commonly known as the tinder-fungus, hoof fungus, tinder conk and tinder polypore or ice man fungus) is a species of fungal plant pathogen found in Europe, Asia, Africa and North America. *Fomes fomentarius* produces very large polypore fruit bodies which are horse's shoe shaped and vary in color from a silvery grey to almost black, though they are normally brown. It grows on the sides of various trees, causing rot. The species typically continues to live on trees long after they have died, changing from a parasite to a decomposer. *Fomes fomentarius* has a fruit body of size between 5 and 45 centimeters across, 3 and 25 cm wide and 2 and 25 cm (0.8 and 9.8 in) thick (Phillips and Roger 1981).

Mushrooms collected were first washed with distilled water. After washing they were shade dried in a well ventilated room. For complete drying, the fruit bodies of mushroom were cut into small pieces and then dried in shade conditions. The dried mushrooms were milled to a fine powder using an electric blender. The mushroom powder was again dried for about 3 h in an oven at 40°C and then stored in plastic polythene bags and kept at room temperature until required for extraction.

3.1.4. Preparation of aqueous extract

The crude aqueous extracts of the selected mushroom was prepared according to the techniques described by Iqbal *et al*. (2006). The powdered mushroom parts (100 g) were extracted with distilled water (500 ml) at 90-100^{o}C in a Soxhlet extractor for 8 h. The aqueous extract was filtered, concentrated and stored at 4 °C until use.

3.2. Experimental design

3.2.1. Experimental animals

Day-old broiler chicks were purchased from local market and screened for coccidial infection. The broiler chicks were reared under standard management practices in the animal house of the Department of Zoology, University of Kashmir, for five weeks. The birds were maintained in a coccidian free atmosphere. The method of housing the

broilers was an intensive deep-litter system. Before the placement of birds, the houses were cleaned, washed, disinfected and provided with saw dust. The ambient temperature in experimental house was maintained at 29°C during the first week and then gradually decreased by 3°C in the third week, and finally fixed at 22°C thereafter. All birds were reared in cages, kept in strictly isolated room. To meet the nutrient requirements of the broiler chicken during the entire experimental period, a complete basal diet was formulated for each of the 2 stages of growth; starter and grower. The diets were formulated to meet the nutrients requirements of broilers as recommended by the National Research Council (NRC, 1994). The chicks were provided with standard coccidiostat free feed. The feed and water was provided *ad libitum* during the study period. Lighting of the environment was provided for 24 hrs. At 22nd day age, the birds were used for experimental purpose. All the birds were tagged to maintain their identity.

On day 22 the body weight of all chicks was taken and grouped into four experimental groups A, B, C and D each having 10 chicks by random allocation. Underweight and weak chicks were excluded from the experiment. The birds in groups A, B and C were inoculated with mixed coccidial oocysts of *Eimeria* species at the rate 3850-4000 sporulated oocysts per bird (Williams, 2001) using insulin syringe introduced directly into the crop of each bird at 22nd day of age. By day 6 post-inoculation (PI), they were treated with mushroom extracts and recommended medicine according to the following schedule:

Group-A: Infected and treated with extract of mushroom (1) in water for 5 consecutive days.

Group-B: Infected and treated with recommended medicine for 5 consecutive days.

Group-C: Infected and un-medicated group.

Group-D: Uninfected and un-medicated group.

Group D served as uninfected and un-medicated control, groups A to C were infected with sporulated oocysts of *Eimeria* on the 22nd day of age. Group C was infected and left untreated. Group B was infected, and treated with the allopathic drug amprolium. The Group A was infected and treated with aqueous extract of *Ganoderma applanatum.* Drinking water was provided *ad-libitum* throughout the entire period of

study. The same experimental design was repeated for second mushroom (*Fomes fomentarius*).

3.2.2. An inventory of birds for procuring infection

An inventory of poultry birds was made for getting coccidian infection in nature. Coccidiosis suspected guts were collected from different poultry farms. All the intestines and caecae were opened and their contents (faeces) were collected in a beaker. The oocysts thus procured were kept in a medium (2.5% Potassium dichromate-$K_2Cr_20_7$) for experimental infection.

3.2.3. Parasite inoculation

Prior to the experimental infection, feacal samples from all experimental groups were collected and examined for any contamination by coccidian infection. All groups were found negative for coccidial oocysts. On 22nd day of age, each group was ready to be inoculated by coccidial oocysts of *Eimeria* species previously obtained from the guts of infected chicks. One ml of oocyst suspension in distilled water was orally inoculated directly into the crop using a flexible plastic tube fitted to 5ml syringe. The sporulated oocysts were given at the dose rate of 3850-4000 oocysts per bird (Williams, 2001).

3.2.4. Determination of weight gain and feed conversion ratio

Performance of broilers was evaluated by recording body weight (BW), daily body weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) during the entire experimental period. Mortality was recorded as it occurred. Weight gain of the broilers was monitored using a weighing balance (made in China by Hana) every morning prior to feeding. The feed: gain ratio per group was determined, where feed: gain per bird=total feed consumption by the birds in a cage divided by weight gain of surviving birds + weight gain of dead birds in the cage. The group with the highest value indicates evidence of depression of feed intake due to infection with *Eimeria*. The broiler mash contained maize, groundnut cake, wheat chaff, rice bran, fishmeal, bone-meal, limestone and premix, giving about 22 % crude protein and 2800 Kcal/kg metabolisable energy. The feeders and drinkers were washed daily using boiling water to reduce the risk of contamination.

3.2.5. Collection of faecal samples and laboratory examination

The birds started shedding oocysts 4 days of post infection. The fecal droppings in each cage were collected on a polyethylene sheet placed on the fecal tray of the cages. The faecal samples were continuously observed after a time interval of 24 hrs, 48 hrs and 72 hrs and severity of infection was confirmed. Diagnosis of *Eimerian* oocysts in faeces is an easy to get an impression of the infection level, direct smear method and both qualitative and quantitative techniques was done to analyze the faecal sample. McMaster's oocyst counting technique was used for counting the coccidian oocysts (Soulsby, 1982). Faeces from each group were thoroughly mixed in plastic bottles using a spatula. One gram of the faecal sample was placed in a sterile bottle and homogenized by mixing with 1ml of saturated sodium chloride (NaCl) solution to make a suspension that was then mixed with 9ml of the salt solution, sieved in gauze wire mesh or muslin cloth, the solid matter was discarded and the filtrate was collected in clean sterile plastic tubes filled to the brim and a cover slip was placed on top taking care to exclude air bubbles. The plastic tubes were allowed to stand upright for 15 min to enable coccidia oocysts to float to the cover slip before examination under a light microscope at 10X and 40X magnifications. A portion of the sample was also used to fill the McMaster counting chamber and allowed to stand for about 15 min to enable oocysts to float and settle at the top of the chamber to facilitate identification and counting of the oocysts under the microscope. Absolute numbers of coccidial oocysts counted per gram of the faecal sample were recorded.

3.2.6. Oocysts counting

To obtain accurate information with regard to severity of an infection, egg counting methods were carried out to determine number of eggs per gram (EPG) of faeces. For this purpose McMaster counting chamber was used. This method is generally used in litter oocyst counting procedures since the percentage of sporulation and oocyst dimensions are not required in this measurement.

3.2.7. McMaster chamber method

The McMaster chamber method is documented by Hodgson (1970), Long and Rowell (1958), and Long *et al.* (1976).

Equipment: Centrifuge, cheesecloth (muslin), beaker, a jar with a lid, or Parafilm, McMaster counting chamber, hand tally counter, 10 or 15ml graduated test tubes, saturated sodium chloride.

Procedure:

1. 10 g of litter was soaked in 100 ml of distilled water for 24 hours at 4°C in a 200 ml beaker that was tightly covered (either with a lid or Parafilm).
2. The beaker was shaken vigorously and the litter was filtered through a single thickness of muslin cloth.
3. A 15 ml centrifuge tube was filled with filtrate to 1 cm from the top and centrifuged for five minutes at a speed that concentrates the solids.
4. The supernatant was discarded. The pellet was resuspended in 100ml of saturated salt solution (NaCl).
5. Two chambers of McMaster counting slide were filled with the suspension with the help of plastic transfer pipette and allowed 3-5 minutes for floatation of oocysts before examination. The oocysts float to the top of the solution, and the total number is counted.

Calculation:

Number of oocysts per gram of litter = n / 0.15 × volume × 0.1

Where n = number of oocysts counted, 0.15 = volume of the McMaster counting chamber, volume = 100 ml of water that the litter is soaked in, and 0.1 = correction for 10 g of litter originally taken.

Therefore, each oocyst counted is equivalent to 67 oocysts per gram of sample. When calculations of oocysts per bird are done, the number of oocysts per gram is divided by the number of birds in the pen to give the number of oocysts per gram per bird.

3.3. Statistical Analysis

The whole data was fed into Microsoft Excel 2010, a computer program (SPSS 11.5 for windows) and Primer software was used for data analysis. The data was represented as mean of replicates followed by standard deviation i.e. Mean ± standard deviation (SD).

1A. *Fomes fomentarius*

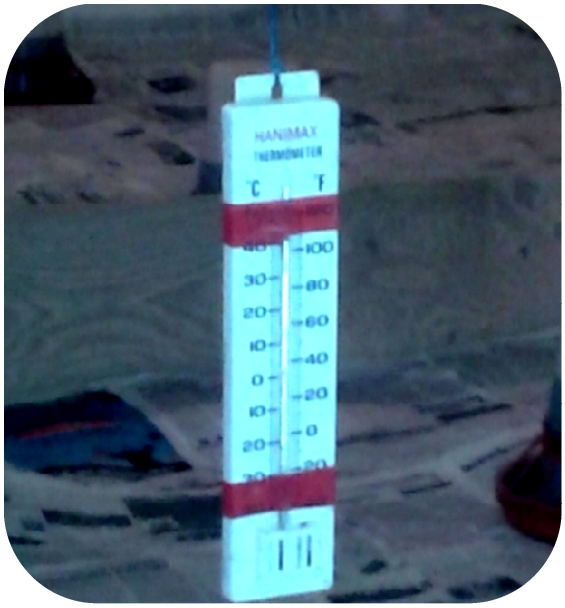
1B. *Ganderma applanatum*

1C. Crushed mushrooms

1D. Soxhlet apparatus

Plate 1. Two mushrooms *Fomes fomentarius* **(1A)** and *Ganoderma applanatum* **(1B)** which are crushed in **(1C)** and extracted using soxhlet apparatus in **(1D)**

2A. Day old chicks

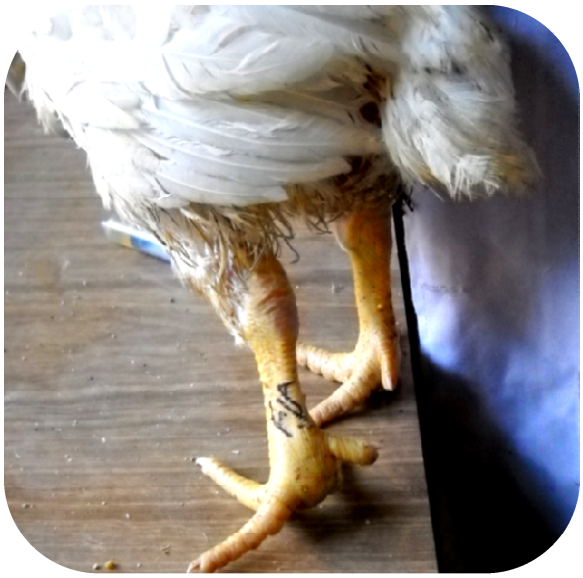


2B. Wall thermometer

2C. Chicks in one group

2D. Inoculation of extract

Plate 2. **(2A)** shows day old chicks and **(2B)** shows wall thermometer while **(2C)** shows chicken in one group and **(2D)** shows inoculation of extract



3A. Chicken labelled on its leg

3B. Weighing chicken

3C. Chick drinker

3D. Chick feeder

Plate 3. **(3A)** shows labelling of chicken **(3B) shows weighing 3(C)** shows chick drinker and **(3D)** shows chick feeder

Chapter: 4

Results & Discussion

Present study was aimed to investigate the efficacy of extracts of some medicinal mushrooms against coccidiosis in broilers. For this purpose two species of mushrooms collected from the different regions of Kashmir valley were screened for their anticoccidial activity in broilers. Coccidiosis remains one of the most economically important diseases in poultry industry. Control of coccidiosis has primarily focused on prophylaxis with anticoccidial drugs in food. Their extensive use has however led to the development of drug resistance, and as a consequence, alternative control strategies against avian coccidiosis have gained importance. The new approaches include use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the chicken immune system (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005). Mushrooms have been known to possess significant pharmacological effects and physiological properties such as bioregulation (immune enhancement), maintenance of homeostasis and regulation of biorhythm, cure of various diseases and prevention and improvement from life threatening diseases like cancer, cerebral stroke and heart diseases (Yuan et al., 1993; Wasser *et al* 1999; Guo *et al.,* 2004; Dalloul and Lillehoj, 2005). Mushrooms are also reported to possess antifungal, anti-inflammatory, antitumor, antiviral and antibacterial activities (Wasser *et al* 1999). Other studies have shown that some mushrooms have polysaccharides that play role in stimulating the activities of many interdependent cell types such as T and B-lymphocytes, macrophages, and natural killer (NK) cells, inducing production and secretion of cytokines and complement (Guo *et al.,* 2003). Some polysaccharides, like those from the herb *Astragalus membranaceus*, and the mushrooms *Lentinus edodes* and *Tremella fuciformis*, were studied more extensively in poultry. It is reported that these mushroom and herb polysaccharides, which were used as feed supplements or vaccine adjuvants, showed antibacterial (Yuan et al., 1993), antiviral (Wei *et al*., 1997; Qu *et al.,* 1998; Liu *et al.,* 1999) and antiparasitic activities (Pang *et al*., 2000). Bioactive compounds or polysaccharides are known to play vital roles in enhancing health; they block colonization of the intestine by pathogens, thereby improving their elimination from the body (Elmusharaf *et al.,* 2006; Guo *et al.,* 2004). With so much diverse pharmacological effects and in view of sufficient literature this study was planned to

unleash the anticoccidial potential of some mushrooms growing in Kashmir valley

For a clear understanding, the observations were divided into following sub headings dealing with various aspects of the study.

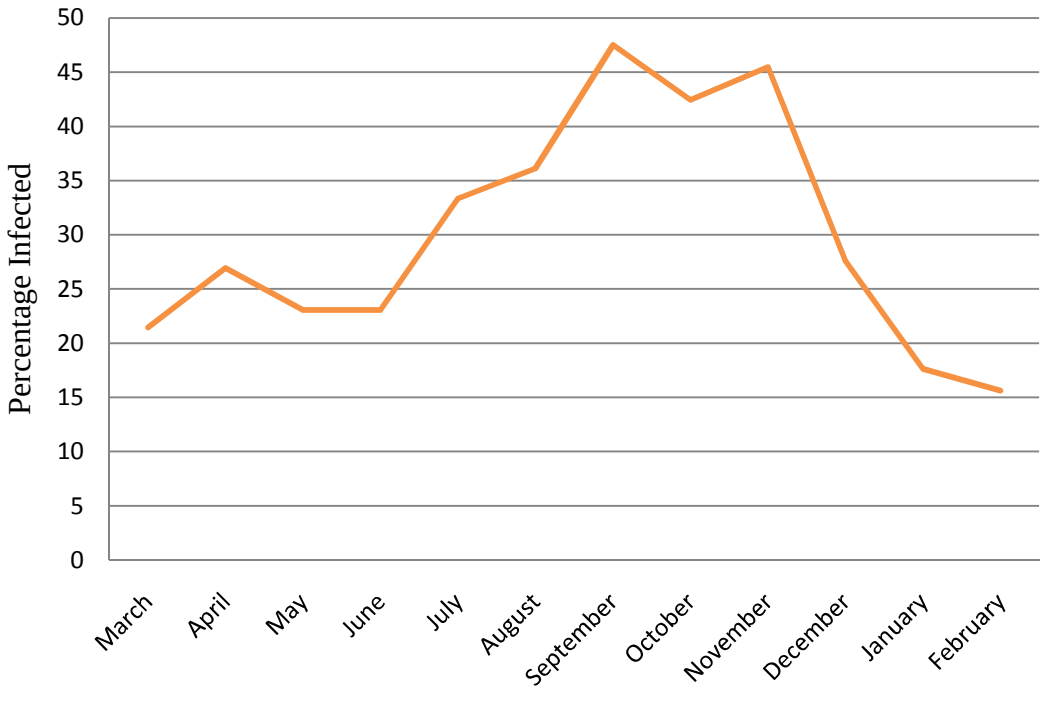
4.1 Prevalence of coccidian infection in poultry birds of Kashmir valley.

A total of 375 gut samples of poultry birds (broilers) were collected from different poultry farms of Kashmir valley. On examination, 112 samples were found to harbor the *Eimeria* parasite. Analysis of the infected samples revealed that coccidiosis was more prevalent in autumn followed by summer, spring and winter. This prevalence pattern of the disease may be correlated with the fact that ambient temperature and relatively higher humidity (>60%) favour the disease by promoting survival and Sporulation of the oocysts (Razmi and Kalideri 2000). During the study period, sampling was done throughout the year to find out the correlation of occurrence of disease in a particular season with distinct environmental conditions. There was, an insignificant difference in prevalence between winter and spring season, although environmental factors have positive impact on the occurrence of disease. The results of present study showed that higher prevalence of diseases was due to the combined effect of high level of relative humidity and ambient temperature, and this was in agreement to the findings of Anderson *et al*. (1976). In the autumn, relatively higher humidity and ambient temperature might be responsible for increased sporulation and thus high prevalence of disease in this season. In summer, prevalence of disease was lower from April to July that might be due to unfavourable climatic conditions (high temperature and low relative humidity) but afterwards prevalence of disease shoots up from August to October when there was decrease in temperature and increase in relative humidity that favoured the developmental stages of coccidial life cycle (Rodriguez-Vivas *et al*., 1996). The findings of the present results are in accordance with the studies of Dar and Anwar (1981) and Khan *et al*. (2006) who also found higher prevalence of coccidiosis. In a study reported from Netherlands, Braunius (1986) and Graat *et al*. (1998) found coccidial infections to occur more often in autumn with high humidity. Results showed that in spite of high relative humidity in winter, coccidial infection was less owing to un-favourable temperature not suitable for sporulation. From these results it was conculuded that coccidiosis is higher in autumn followed by summer, spring and winter.

Table 3. Prevalence of coccidian infection in poultry birds of Kashmir valley from Jan-Dec 2012

Month	Season	Examined	Infected	Percentage Infected	Mean ± SD
March	Spring	28	6	21.43	23.81 ± 2.81
April		26	7	26.92	
May		39	9	23.08	
June	Summer	26	6	23.08	30.84 ± 6.86
July		30	10	33.33	
August		36	12	36.11	
September	Autumn	40	19	**47.50**	**45.12 ± 2.55**
October		33	14	**42.42**	
November		22	10	**45.46**	
December	Winter	29	8	27.59	20.29 ± 6.40
January		34	6	17.65	
February		32	5	15.63	
Total		**375**	**112**	**29.87%**	**P<0.05**

Fig. 3. Prevalence of coccidian infection in poultry birds of Kashmir valley



4.2. To observe the effect of *Fomes fomentarius* extract against coccidiosis in broilers (Experiment 1).

Following parameters were studied:

4.2.1. Oocyst per gram (OPG) counts

The OPG counts of different groups of chicken are represented in (Table 4, Fig 4). The highest oocyst count per gram of faeces (OPG) was recorded in group C as it was untreated group. Prior to treatment at 26th day the oocyst output of birds was 5010.71 ± 28.029 oocysts/g faeces (group A), 4879.40 ± 25.827 oocysts/g faeces (group B) and 5187.21 ± 23.825 oocysts/g faeces (group C). The faeces of uninfected group D were free of coccidial oocysts. After treatment the oocysts detected in the *F. fomentarius* treated group (A) on 27th day had reduced significantly in number (2800.58 ± 16.920 oocysts/g faeces) compared to un-treated group (C) which showed increase in oocysts released (5730.42 ± 26.150 oocysts/g faeces). By 28th day, the oocysts released in A and B group had reduced to 986.0 ± 11.230 and 15.36 ± 1.129 respectively and by day 29 the birds in these groups were almost free of infection (group A 210.34 ± 6.099) (group B 3.26 ± 0.053), while group C has shown continued discharge of oocysts in high number.

4.2.2. Body weight gain records and Feed conversion ratio

The impact of oral administration of sporulated coccidial oocysts on body weight gain of different groups of chicken followed by administration of *F. fomentarius* extract are represented in (Table 5, Fig. 5). The mean initial weight of chicks for all groups was almost similar which was recorded on day 1 and day 22nd. Among the treated groups the significant improvement in body weight was recorded in group B. Chickens of group A gained the next highest body weight on the same day. The results further showed that infection with coccidial oocysts result in the decrease of feed intake of birds in all the infected groups, but this was followed by a compensatory increase in feed intake in group A and B after treatment. Feed conversion ratio was recorded higher in group C as compared to all the other treated groups. The mean weight gain of the birds in group C at day 35 was also significantly lower (1201.34 ± 12.981 grams) than other treated groups.

Table 4. Oocyst output of broilers treated in different groups.

Oocyst output per gram of faeces	Different treatment groups			
	Group A	Group B	Group C	Group D
After infection at 26th day	5010.71 ± 28.029	4879.40 ± 25.827	5187.21 ± 23.825	0
After treatment at 27th day	2800.58 ± 16.920	2274.91 ± 19.345	5730.42 ± 26.150	0
After 2 days of treatment at 28th day	986.01 ± 11.230	15.36 ± 1.129	6088.03 ± 23.384	0
After 3 days of treatment at 29th day	210.34 ± 6.099	3.26 ± 0.053	6552.27 ± 28.196	0
Significance	**	***	NS	NS

(* less significant; ** more significant; *** highly significant, NS Not Significant)

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 4. Reduction in oocyst output of different treatmet groups

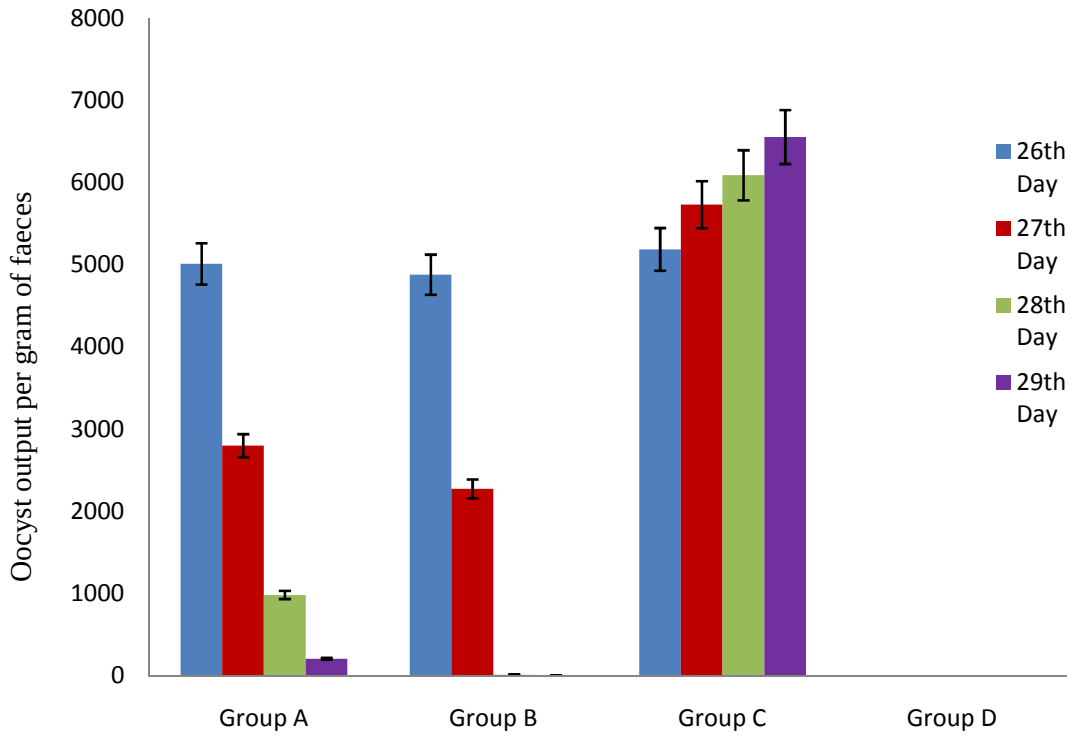


Table 5. Mean weight gain/Group (in grams) of broilers treated in different groups.

Parameters	Age in days	Group mean weight gain (in grams)			
		Group A	Group B	Group C	Group D
Initial weight	1	37.55 ± 0.541	36.98 ± 0.643	36.8 ± 0.585	38.04 ± 0.621
At pre-infection	22	590.84 ± 2.85	585.32 ± 2.69	586.28 ± 3.15	595.32 ± 2.52
At infection time	24	610.92 ± 3.762	601.66 ± 3.20	602.49 ± 2.983	610.45 ± 3.045
Before treatment	26	761.33 ± 4.322	796.57 ± 4.172	720.32 ± 4.165	828.35 ± 3.873
Three days after the treatments	28	1022.11 ± 8.643	1105.73 ± 7.653	984.9 ± 8.960	1244.76 ± 7.924
Seven days after the treatments	35	1350.41 ± 12.402	1470.25 ± 13.122	1201.34 ± 12.981	1570.86 ± 11.137

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 5. Mean Weight gain of chicks in different treatment groups

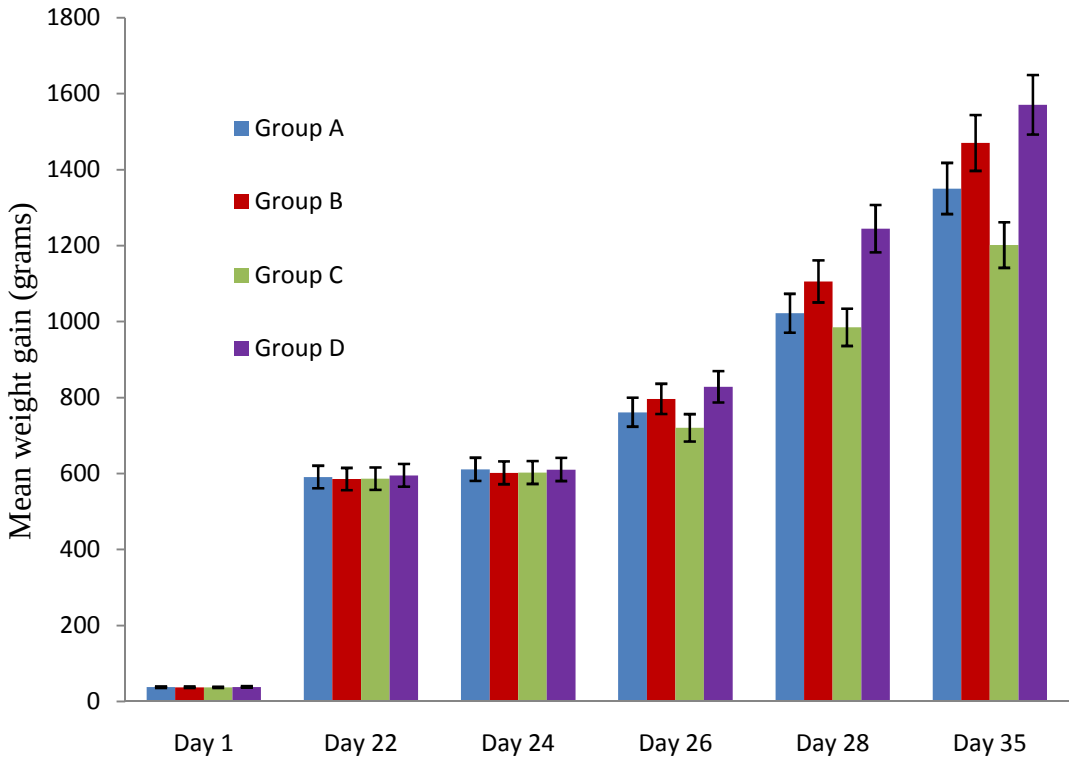
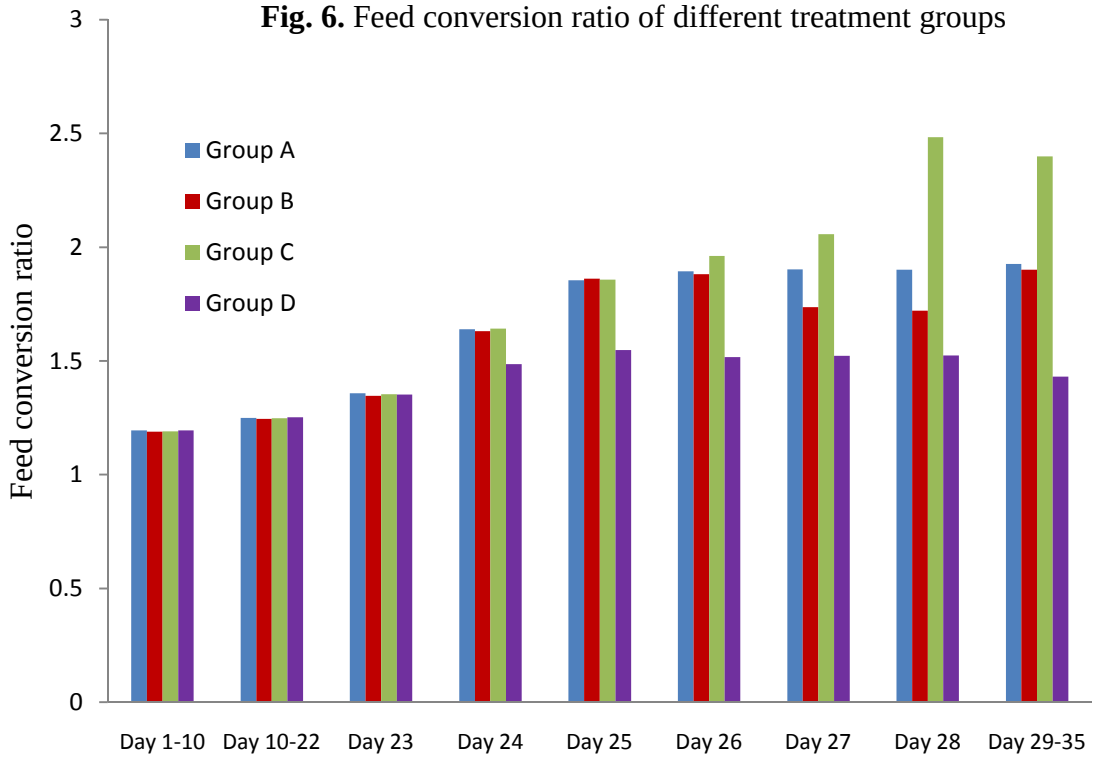


Table 6. Feed Conversion ratio of broilers treated in different groups.

Parameters	Days	Feed Conversion Ratio = Feed consumed / Weight gained			
		Group A	Group B	Group C	Group D
Not infected	1-10	**1.195**	**1.189**	**1.191**	**1.194**
	11-22	**1.250**	**1.246**	**1.248**	**1.252**
Infected	23	**1.358**	**1.347**	**1.354**	**1.352**
	24	**1.639**	**1.631**	**1.642**	**1.486**
	25	**1.855**	**1.862**	**1.858**	**1.548**
During treatment	26	**1.894**	**1.881**	**1.961**	**1.517**
	27	**1.902**	**1.736**	**2.057**	**1.523**
	28	**1.901**	**1.721**	**2.483**	**1.524**
After treatment	29-35	**1.927**	**1.901**	**2.399**	**1.431**

Group A = Infected and treated with aqueous extract of *Fomes fomentarius* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 6. Feed conversion ratio of different treatment groups



4.3. To observe the effect of *Ganoderma applanatum* extract against coccidiosis in broilers (Experiment 2).

Following parameters were studied:

4.3.1. Oocyst per gram (OPG) counts

The OPG counts of different groups of chicken are represented in (Table 7, Fig. 7). The highest oocyst count per gram of faeces (OPG) was recorded in group C as it was untreated group. Prior to treatment at 26th day oocyst output of birds was 7300.68 ± 32.792 oocysts/g faeces (group A), 7658.12 ± 31.652 oocysts/g faeces (group B) and 7013.89 ± 32.048 oocysts/g faeces (group C). The faeces of uninfected group D were free of coccidial oocysts. After treatment at 27th day the oocysts detected in the *G. applanatum* treated group (A) had reduced significantly in number (4700.92 ± 24.414 oocysts/g faeces) compared to untreated group C which showed a significant increase in oocysts released (5709.61 ± 26.285 oocysts/g faeces). By day 28, the oocysts released in A and B group had reduced to 2886.67 ± 16.230, 196.09 ± 3.864 respectively and by day 29 the oocyst output in groups was (1490.58 ± 13.652 group A), (16.44 ± 0.701 group B) while group C continued to discharge of oocysts in high number.

4.3.2. Body weight gain records and feed conversion ratio

The mean initial weight of chicks for all groups was almost similar which was recorded on day 1 and day 22nd. Among the treated groups the significant improvement in body weight was recorded in group B. Chickens of group A gained the next highest body weight on the same day. The results further showed that infection with coccidial oocysts results in the decrease of feed intake of birds in all the infected groups, but this was followed by a compensatory increase in feed intake in group A and B after treatment. Feed conversion ratio was higher in group C as compared to all the other groups. The mean weight gain of the birds in group C at day 35 was also significantly lower (1220.77 ± 12.739) than other treated groups (Table 8, Fig. 8).

Table 7. Oocyst output of broilers treated in different groups.

Oocyst output per gram of faeces	Different treatment groups			
	Group A	Group B	Group C	Group D
After infection at 26th day	7300.68 ± 32.792	7658.12 ± 31.652	7013.89 ± 32.048	0
After treatment at 27th day	4700.92 ± 24.414	3178.74 ± 23.259	5709.61 ± 26.285	0
After 2 days of treatment at 28th day	2886.67 ± 16.230	196.09 ± 3.864	6057.32 ± 29.497	0
After 3 days of treatment at 29th day	1490.58 ± 13.652	16.44 ± 0.701	6200.45 ± 30.336	0
Significance	*	***	NS	NS

(* less significant; ** more significant; *** highly significant, ^{NS} Not Significant)

Group A = Infected and treated with *Ganoderma applanatum* extract at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 7. Reduction in oocyst output of different treatment groups

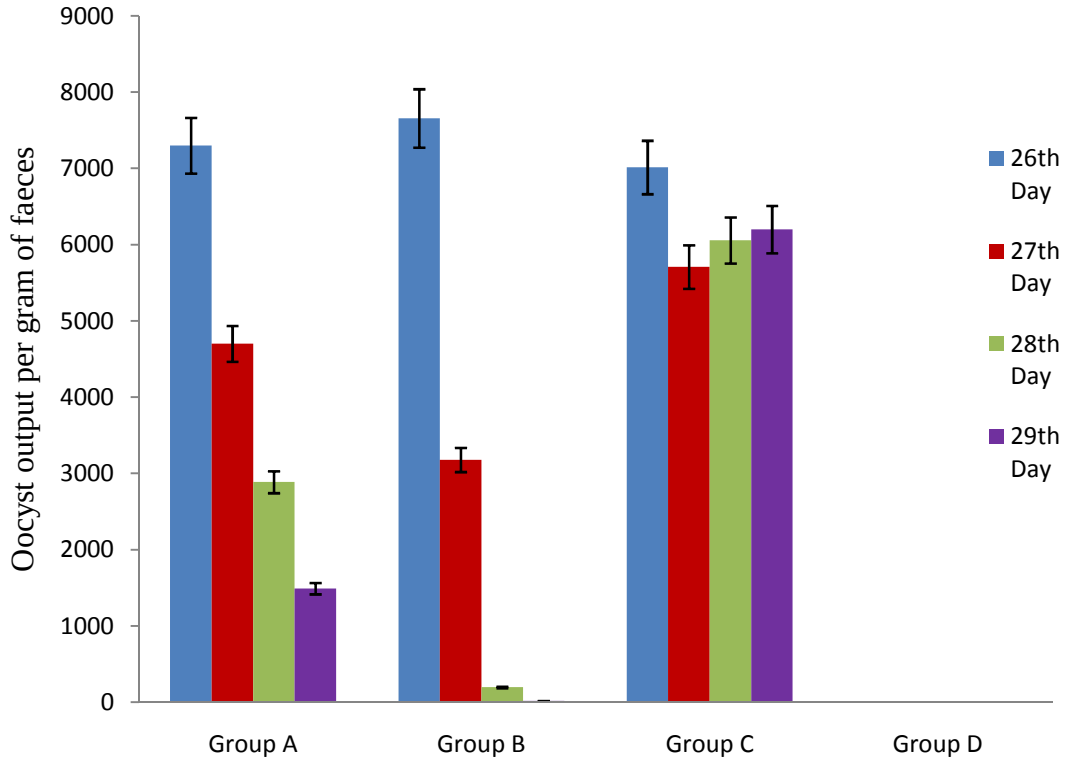


Table 8. Group mean weight gain (in grams) of broilers treated in different groups.

Parameters	Age in days	Group mean weight gain (in grams)			
		Group A	Group B	Group C	Group D
Initial weight	1	39.33 ± 0.493	38.72 ± 0.562	37.88 ± 0.581	38.12 ± 0.620
At pre-infection	22	572.21 ± 2.72	580.95 ± 2.69	564.85 ± 2.27	583.36 ± 2.31
At infection time	24	597.87 ± 2.704	604.54 ± 3.171	607.64 ± 2.962	625.75 ± 3.105
Before treatment	26	761.54 ± 4.143	816.17 ± 4.864	720.29 ± 4.295	836.61 ± 5.169
Three days after the treatments	28	1012.74 ± 8.672	1165.4 ± 8.973	984.29 ± 8.380	1244.52 ± 9.657
Seven days after the treatments	35	1318.59 ± 12.004	1496.63 ± 13.597	1220.77 ± 12.739	1570.36 ± 14.640

Group A = Infected and treated with *Ganoderma applanatum* extract at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

Fig. 8. Mean Weight gain of chicks in different treatment groups

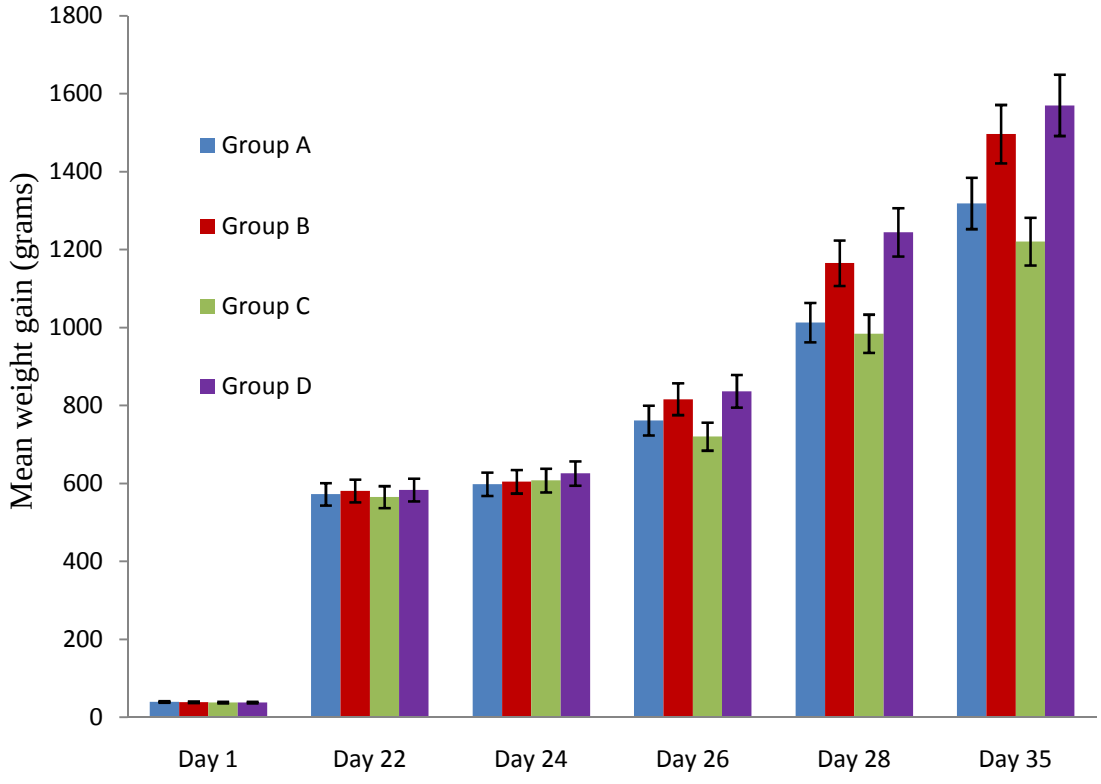


Table 9. Feed Conversion ratio of different treatment groups.

Parameters	Days	Feed Conversion Ratio = Feed consumed / Weight gained			
		Group A	Group B	Group C	Group D
Not infected	1-10	**1.188**	**1.187**	**1.190**	**1.192**
	11-22	**1.247**	**1.240**	**1.251**	**1.243**
Infected	23	**1.344**	**1.349**	**1.352**	**1.340**
	24	**1.645**	**1.656**	**1.639**	**1.452**
	25	**1.863**	**1.855**	**1.852**	**1.530**
During treatment	26	**1.890**	**1.879**	**1.896**	**1.527**
	27	**1.920**	**1.732**	**2.126**	**1.518**
	28	**1.937**	**1.725**	**2.464**	**1.522**
After treatment	29-35	**1.925**	**1.738**	**2.390**	**1.361**

Group A = Infected and treated with aqueous extract of *G. applanatum* at 1,000 mg/kg body weight, **Group B** = Infected and treated with amprolium, **Group C** = Infected but not treated, **Group D** = Neither infected nor treated

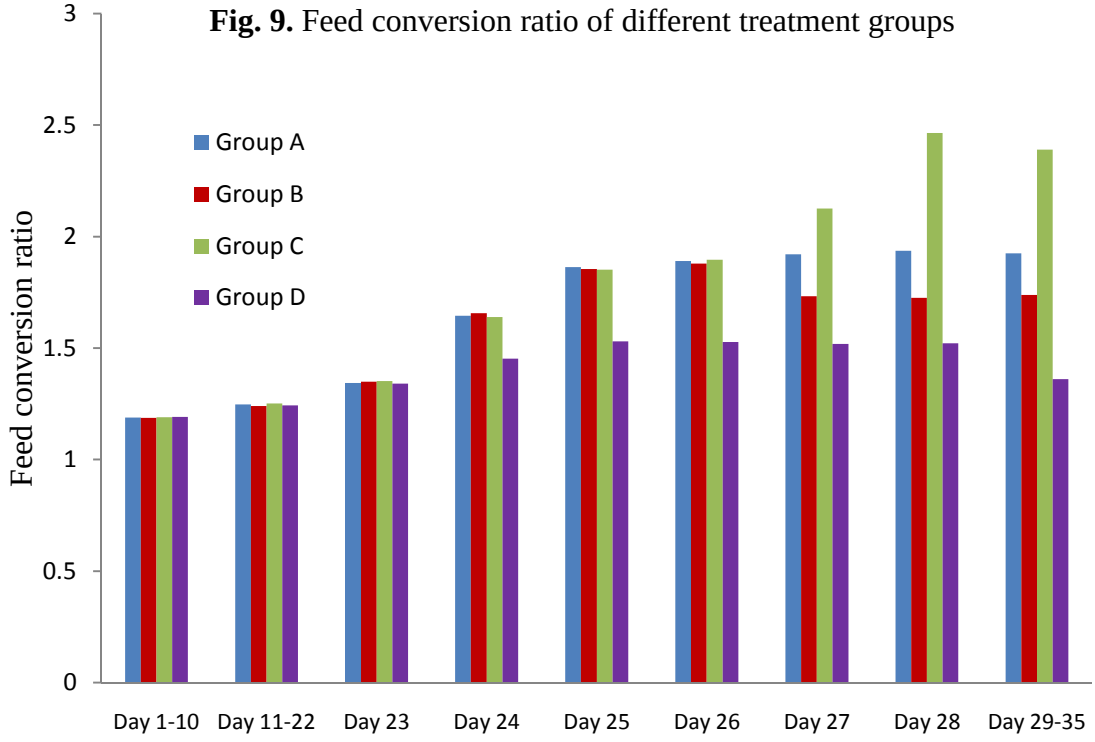


Fig. 9. Feed conversion ratio of different treatment groups

In vivo anticoccidial effects of aqueous extract of two mushrooms *G. applanatum* and *F. fomentarius* against coccidiosis in broilers were evaluated on the basis of oocysts per gram of feaces, weight gain and feed conversion ratio and compared with the reference drug Amprolium. Polysaccharide β-glucan isolated from mushrooms is well known for its immune modulatory properties, in addition many secondary metabolites, and extra cellular secretions of mycelia have antibacterial and antiviral properties. Also the exudates from mushroom mycelia are active against *Plasmodium falciparum* (protozoa that cause malaria) and other microorganisms (Sonawane *et al.,* 2012). Historically, the severity of *Eimeria* infection has been assessed by reduced body weight gain and the excretion of fecal oocysts (Idris *et al.,* 1997).

The experimental infection of the broiler chickens with coccidial oocysts showed clinical signs of weakness, reduced appetite, diarrhoea, and presence of oocysts in faeces. The experimental trials in all the infected birds showed a significant reduction in faecal oocyst output in birds that were treated with either aqueous extract of *G. applanatum* or *F. fomentarius* or amprolium. However the lowest OPG was recorded in amprolium treated groups in both experiments indicating the highest prophylactic efficacy among all groups. The results in terms of use of aqueous extract of *F. fomentarius* and *G. applanatum* to reduce oocysts of coccidia in broilers was in full agreement with Conway, *et al.,* (1993) who studied the *e*ffects of different levels of oocysts inocula of *Eimeria acervulina, E. tenella* and *E. maxima* on plasma constituents, packed cell volume, lesion scores and performance in chickens and Elmusharaf *et al.,* (2006) who investigated the effect of a Manna-oligosaccharide (MOS) preparation on *Eimeria tenella* infection in broiler chickens. Moreover the results of this study are also in agreement with Wills, *et al.,* (2010), they strongly suggest that a diet supplemented with 5% FMG as an alternative control method in reducing *Eimeria* oocyst numbers during grow out. Likewise, Allen *et al.* (1998) reported that the herb *Artemesia annua* reduces oocyst yield. These results are also in line with Misra, *et al.* (1993), who reported that Zycox (herbal anticoccidial) is effective to reduce faecal oocysts output. Large number of oocysts were produced in all the infected groups; however, the oocyst count of treated groups appeared to be significantly lower than in the remaining groups. In this study the rate of oocyst shedding increased slowly after infection then it decreased. This happened because at

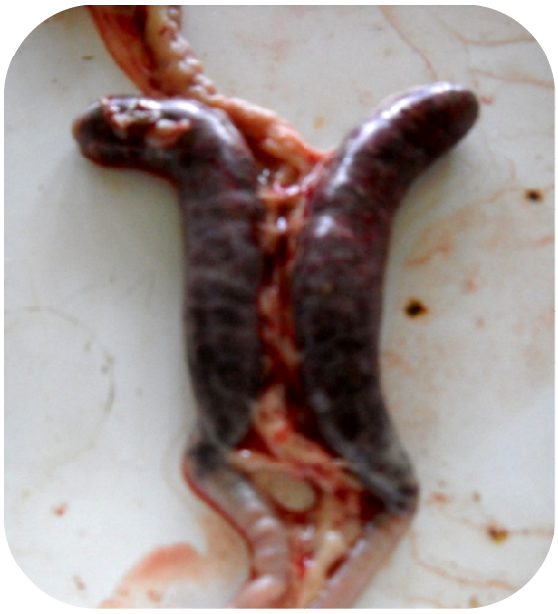
the beginning of infection, chickens immune system could not lower the oocyst output, but after few weeks, immunity level went up and decrease the oocyst output (Mathis, 1999 and Weppelman *et al.,* 1997)

The highest feed conversion ratio observed in the infected, untreated birds (2.399, 2.390) provides an evidence of reduction of feed intake due to infection with coccidian oocysts. The highest feed conversion ratio reported in infected broilers resulted in significant reduction in the body weight. The study revealed that groups of birds not infected with coccidial oocysts consume more feed, while infected groups showed lower feed intake due to coccidial stress. Hayat *et al.,* (1991); Ogbe *et al.,* (2009) and Conway *et al.,* (1993) also reported that a significant reduction in body weight in broilers infected with oocysts of *E. tenella*. The effect of infection on growth performance may be related to the degree of the infection. Under conditions of more severe infection with *Eimeria*, weight gain is generally reduced (Johnson and Reid, 1970; Conway *et al*., 1993; McDougald, 2003; Chapman *et al.,* 2004).

The results of the present work showed in first experiment the birds of group A, infected and treated with aqueous extract of *F. fomentarius* extract had significantly higher mean weight gain (1350.41 ± 12.402 g) and lower feed conversion ratio (FCR) (1.927), whereas birds of group C, infected but not treated gained lowest weight (1323.9 g) and highest FCR (2.399). Similarly in other experiment significant mean weight gain and FCR was recorded in birds of group A, (infected and treated with aqueous extract of *G. applanatum*, weight gain=1318.59 ± 12.004, FCR=1.925. It seemed that both these mushrooms acted as growth promoter. Brisibe *et al.,* (2008) observed higher weight gain in broilers on diets containing dried *Artemesia annua* leaves. Similar results were also reported by Miao, *et al.,* (2000). The poorest FCR was observed in birds which were infected but non-medicated. These results are supported by Voeten *et al.,* (1988) who found that coccidiosis adversely affected growth and feed conversion.

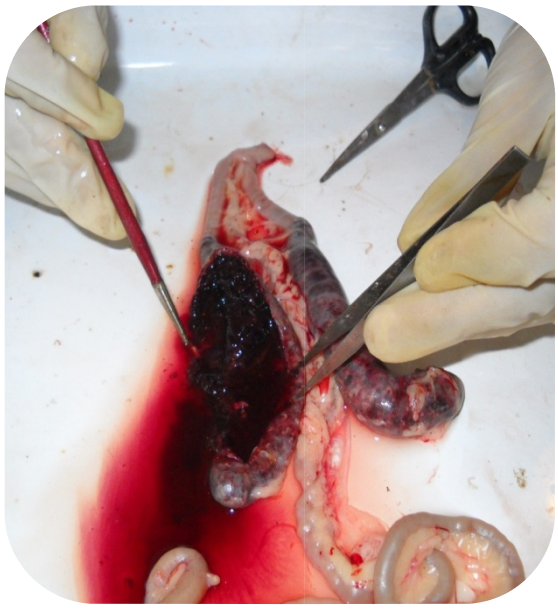
It has already been found that the mushroom extract and amprolium stimulate appetite, so the broilers that were treated with them ate more and improved in weight gain better than those un-treated birds. The broilers that were not infected but treated with either amprolium or *G. applanatum* or *F. fomentarius* performed better in terms of weight gain than those that were infected and treated. From these observations it is apparent that wild mushrooms contain compounds that are active against coccidia.

Bioactive compounds or polysaccharides are known to play vital roles in enhancing health; they block colonization of the intestine by pathogens, thereby improving their elimination from the body (Elmusharaf *et al.,* 2006; Guo *et al.,* 2004., Hughes, *et al.,* 1958). Some biologically active compounds or organic acids, resins, and glycosides which include steroid and triterpenoid saponins are known to have therapeutic uses against microbes and parasites (Anon, 2006; Die *et al.,* Guo *et al.,* 2004; Hobbs, 1995). The mushrooms used in this study may also possess these active compounds. Other studies have shown that some mushrooms have polysaccharides that play a role in stimulating the activities of many interdependent cell types such as T and B-lymphocytes, macrophages, and natural killer (NK) cells, inducing production and secretion of cytokines and complement(Guo, *et al.,* 2004). Other mushrooms (e.g. *Fraxinella, Boletus* and *Lactarius* spp.) have also been reported to prevent intestinal coccidiosis in poultry (Guo *et al.,* 2004*;* Harkonen, 1998; Pang, *et al*). Some mushrooms contain chemical substances that enhance the immune response and control certain parasitic and viral diseases (Guo *et al.,* 2004*;* Oei, 2003; Wachtel *et al;* Wasser 2002; Zakhary, et al., 1983). However, the active principles and the mechanisms of action of these mushrooms have not been fully elucidated, and should be the subject of future studies. This study showed that treatment with either *G. applanatum* or *F. fomentarius* resulted in a marked reduction in the number of coccidian oocysts shed in the faeces, leading to improved weight gain. The results confirmed the virulence of coccidian oocysts and the effectiveness of both amprolium and *G. applanatum* and *F. fomentarius* extract against coccidian oocysts. Hence, these mushrooms can be used as a better natural alternative method for control of coccidial infection in broilers and can be used in free range poultry. Further studies may be helpful in isolating the active components effective the against coccidiosis from these mushrooms and to know their mechanism of action on different stages of coccidia.

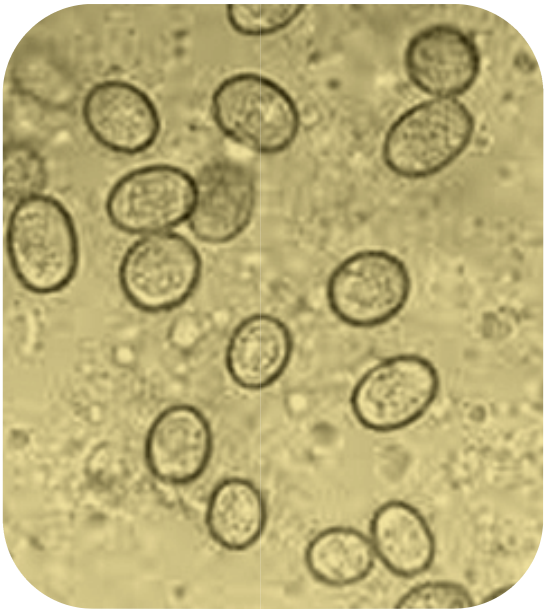


4A. Eimeria infected caecum

4B. Dissection of chicken intestine



4C. Blood inside caecum



4B. Eimerian oocysts

Plate 4. **(4A)** shows infected caecum **(4B)** shows dissection of chicken intestine **(4C)** shows blood in caecum and **(4D) shows** Eimeria oocysts

Chapter: 5

Summary & Conclusions

Coccidiosis is a parasitic disease that is a constant health problem, especially in intensive poultry industry. It is the most important infectious poultry disease, as far as economy is concerned. Coccidiosis is a global disease and costs on yearly basis, for prophilaxis, as well as therapy exceed two billion Euros (Dallouil and Lillehoj, 2006). One of the main expenses faced by the industry is loss associated with poultry diseases, including costs of vaccination, prevention, treatment, reduction in weight gains, and mortality.The new approaches include the use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the chicken immune system (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005). Mushrooms have been known to possess significant pharmacological effects and physiological properties such as bioregulation (immune enhancement), maintenance of homeostasis and regulation of biorhythm, cure of various diseases and prevention and improvement from life threatening diseases such as cancer, cerebral stroke and heart diseases (Yuan et al., 1993; Wasser *et al* 1999; Guo *et al.*, 2004; Dalloul and Lillehoj, 2005).

With this background in mind the study was planned with following aims and objectives:

1. Study of coccidian infection in broilers.
2. Experimental infection of broilers with coccidian oocysts procured from infected poultry birds.
3. Oocyst count per gram of faeces after infecting the chicken.
4. Oral administration of mushroom extract.
5. To observe the effect of medicinal mushroom extract on coccidiosis on the basis of oocyst count per gram of faeces, weight gain and feed conversion ratio.
6. Comparison of efficacy of medicinal mushroom extracts versus the drug recommended for coccidiosis.

The study of prevalence of coccidian infection in broilers is the first step of the present research work. In fact it provides the basic foundation for any parasitic control measures. A total of 375 gut samples of poultry birds (broilers) were collected from different poultry farms of Kashmir valley. On examining 375 gut samples 112 were found to harbor the *Eimeria* parasite. In the present study, data showed that coccidiosis was more prevalent in autumn (August 47.5%, September 42.42% and October 45.46%) followed by summer, spring and winter. For observing the effect of medicinal mushroom extracts on coccidiosis two experiments (3 replicas of each) were conducted to study the effect of *Ganoderma applanatum* aqueous extract, *Fomes fomentarius* aqueous extract in coccidian infected broilers. At 22nd day of age in both experiments the birds were randomly allocated to 4 treatment groups of 10 chicks each. In both experiments group A, B and C were infected with coccidian oocysts at the rate of 3850-4000 oocysts per bird and group D was uninfected control group. At 27th day the broilers in group A of respective experiments were treated with aqueous extract of *F. fomentarius* (exp 1) and *G. applanatum* (exp 2) and those in B group with amprolium at the rate of 200mg/ml. Oocyst output, mean body weight gain and feed intake were monitored. The result showed that all the infected birds in groups A, B and C had clinical signs of weakness and reduced appetite on day 4 post-infection. After treatment at 27th day the oocysts detected were considerably reduced in both treated groups A and B of both experiments and slightly higher in the untreated group C. The faeces of the uninfected control groups D were normal and free of coccidial oocysts. After treatment at 29th day very little number of coccidial oocysts were found in the faeces of birds. Infected, untreated birds showed a slight drop in feed intake and weight gain from 22nd - 35 day of age. The final mean weight gain recorded in the treated groups A and B was comparable to that of the uninfected birds, while it was lower in the untreated group C. The feed conversion ratio in both experiments was higher in group C than in the other groups. This study showed that treatment with *G. applanatum* and *F. fomentarius* results in a marked reduction in the number of coccidial oocysts shed in the faeces, leading to improved weight gain. The results confirmed the pathogenicity of the coccidial oocysts and the effectiveness of both amprolium and mushroom extracts against coccidian parasites.

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