EFFECT OF DIETARY INCLUSION OF AZOLLA (*Azolla pinnata*) ON PRODUCTION PERFORMANCE OF BROILER CHICKEN

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2008

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DECLARATION

I hereby declare that this thesis, entitled **"EFFECT OF DIETARY INCLUSION OF AZOLLA (*Azolla pinnata*) ON PRODUCTION PERFORMANCE OF BROILER CHICKEN"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"EFFECT OF DIETARY INCLUSION OF AZOLLA (*Azolla pinnata*) ON PRODUCTION PERFORMANCE OF BROILER CHICKEN"** is a record of research work done independently by **BALAJI. K.**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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ACKNOWLEDGEMENTS

I may be given credit for having blazed the trail but when I took at the subsequent developments I feel the credit is due to others rather than to myself.

- Alexander Graham Bell

On this day I met the finale of my endeavor I express my deep hearted gratitude and indebtness to my esteemed advisor ***Dr. A. Jalaludeen,*** *Professor and Head, Director i/c, Centre for Advanced Studies in Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, for his wholehearted support, valuable advice and guidance rendered throughout the course of my post graduate study. I am overwhelmed by him expertise and brilliance.*

I am extremely thankful to ***Dr. P.A. Peethambaram,*** *Professor, Centre for Advanced Studies in Poultry Science as a member of advisory committee, for his valuable help, constant encouragement, constructive creticism and never failing support shown at every stage of this research work.*

The search of suitable word to express my thanks is still not over as it is beyond the power of my expression to ***Dr. R. Richard Churchil,*** *Associate Professor, AICRP on poultry for eggs, College of Veterinary and Animal Sciences, Mannuthy, member of the advisory committee, for making me rethink many of the issues that I thought I had answered and thus enabling me to arrive at a better understanding of them. His critics helped me recognize when I was skirting the hard questions. I am particularly greatful for his emphasis on simpilicity and elegance in life.*

*I would thank **Dr. A. Kannan** Associate Professor (SS) CPPR, College of Veterinary and Animal sciences, Mannuthy and member of the advisory committee, for his constant monitoring of my performance, solid support in the planning and execution of research and careful direction of my curricular activities with extreme charisma and pragmatism.*

*As such it is imperative to thank **Dr. Narayanankutty,** Senior Scientist, AICRP on Poultry for eggs and **Dr. Amirtha Viswanathan,** Professor, Centre for Advanced Studies in Poultry Science, as part of the work would not have been possible without a great deal of support from her, she was quite but always insightful. Her approach deserves the highest respect I can give.*

*I owe my sincere gratitude to **Dr. P. Anitha,** Associate Professor, and Centre for Advanced Studies in Poultry Science, for her constant encouragement, motivation, valuable guidance, timely help and moral support.*

*I humbly place on record my respect and gratitude to **Dr. Leo Joseph,** Professor and Head, University Poultry farm, for his perspicuous guidance during the tenure of my work.*

*I greatfully acknowledge **Dr. A.D. Mercy,** Professor and Head, Depatment of Animal Nutrition and **Dr. Lalitha Kunjamma,** Professor and Head, Depatmet of Pathology, for providing the disease diagnosis laboratory facilities for my research work.*

*I wish to place on record my sincere thanks to **Dr. G. Deepa,** Assistant Professor, and Centre for Advanced Studies in Poultry Science, for her pleasant co-operation.*

*I extent my heart felt thanks to **Dr. Binoj Chacko,** Teaching Assistant, Department of Poultry Science for his continuous help.*

My two years at COVAS were made much more enjoyable by the companionship of my collegues **Drs. Girish Kumar, Chandini Herman, Shamna T.P. and Bhadra P.V.**, I feel lucky to share my post graduate studies with them.

I sincerely acknowledge the staff of our Department **Mrs. Vilasini, Mr. Ramesh, Mr. Raffi** and **Miss. Indu** for their love and support.

Special thanks to **Dr. Kishore Kumar,** for the brotherly and wholehearted support rendered by him.

I greatfully acknowledge **Dr. S. Senthilkumar,** Assistant Professor, TANUVAS and Ph.D Scholar, for his constant valuable help.

The help rendered by **Smt. Sujatha,** Associate Professor and Head, Department of Statistics, in the statistical interpretation of results is greatfully acknowledged.

I owe a flow of thanks to **Ms. Rathika,** central instrumentation lab for her personal help.

I express my diction of thanks to the staff of RFH for the help rendered in my research work particularly **Ramani** and **Deepthy.**

I am greatful to my dearly loved seniors **Dr. Nitteen, Dr. Simi, Dr. Vijay Singh, Dr. Raseena Karim, Dr. Preethymol** and **Dr. Preetha.**

I take great pleasure in thanking **Dr. E. Nanu,** Dean i/c, Faculty of Veterinary and Animal sciences, for providing me the facilities for my research work.

I greatfully acknowledge the wholehearted support and help rendered by **Drs. K.Unni Krishnan, P. Kanaran, P. Selvakumar, Bibu John, Albert, Rojan, Midun, Ganesh, Prince, Nishant, Aslam, Rajendra** and **Girish** in assisting me during my work and their timely help.

*The invaluable help rendered by my beloved juniors **Drs. Suraj, Nimila** and **Arùnima** are duly acknowledged.*

*I am in short of words to express my deep sence of greatfulness for the understanding, love and encouragement of **Dr. Binoj Chacko** and **Dr. Kishore Kumar** who remaining my confident and very good seniors.*

*I was particularly fortunate to have had respectful seniors **Drs. Rishi, Senthil (P.K), Shanmugam, Vikram, Rajaganapathy, Vivek, Sekar, Babu, Prajith** and **Viswanathan (mess master)** in the PG hostel, with whom I have shared many hours of interesting discussions. They helped me stay in hostel as an enjoyable and memorable one.*

*I would like to thank **Dr. Praveena Churchil** for her care and kindliness forever.*

*No words of thanks could be enough to those **mute creatures,** which have laid down their invaluable life for my work. I am deeply indepted from bottom of my heart to those creatures.*

*A very big thanks goes of course to my **loving parents** for instilling the love of learning in me and to my brother and sister for accompanying me through the ups and downs of my life. They helped me not only in charting a great course but also in providing me with the skills I need to conquer the road ahead.*

*Without the help of these nothing would have been possible. I bow before the **almighty**, for the interminable blessings that have helped me in every stage of my life.*

Balaji. K

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INTRODUCTION

1. INTRODUCTION

Poultry development has taken a quantum leap in the last three decades emerging from a near backyard practice to a venture of industrial promotion. In India broiler industry is the fastest expanding component of agriculture registering 15 per cent growth rate per annum and the annual growth rate of broiler production is much higher in comparison with many other developing countries.

The broiler meat production in India has increased from 1.2 million tones to 2.2 million tones during the period from 2002 to 2007, according to USDA. Despite such growth, the percapita consumption of meat is too low, approximately 3.5 to 4 kg of meat including 2.2 kg of chicken meat as against ICMR recommendation of 11 kg meat per person annually. Considering the feed conversion efficiency and low cost of production broiler farming holds the promise to bridge this deficit.

The increase in broiler production is in direct competition with growing human population for high energy cereal grains. There is also a shortage for oil cakes and other protein supplements, because of increased export for augmenting foreign exchange resources. There is inadequate supply of poultry feed ingredients both in terms of quantity and quality due to urbanization and decreased land availability. The wide gap between the availability and requirement of feed materials may have an adverse effect on broiler enterprise.

Greater emphasis has been given during the past two decades to identify the new feedstuffs to widen the feed resource base, thereby to improve the efficiency and economic viability of broiler production. Inspite of extensive research on utilization of unconventional feedstuffs of agricultural, industrial, animal and aquatic origin there is only limited scope on actual usage of such materials in poultry rations, because of seasonal and scattered availability, variable nutrient quality, poor storage conditions and the presence of antinutritional factors or toxins.

Recently there is an increased emphasis in the use of aquatic plants in poultry rations because the protein and other nutrient content in aquatic plants are comparable to certain leguminous plants. Aquatic plants offer relatively cheap alternative feedstuffs and have many potential uses such as human food, animal feed, compost and biofertilizers.

Among the aquatic plants floating fresh water fern, *Azolla pinnata* is a good source of protein and it contains almost all essential amino acids, minerals like iron, calcium, magnesium, potassium, phosphorus, manganese etc., apart from appreciable quantity of vitamin A precursor beta carotene and vitamin B_{12}. It is capable of assimilating atmospheric nitrogen due to the presence of symbiotic algae in its leaves. It is also found to contain probiotics and biopolymers (Pillai *et al.*, 2004).

Aquatic plant species accumulate secondary plant compounds and therefore offer a greater potential than other types of leaf protein sources for monogastric animals. The water fern Azolla, which grows in association with the blue-green algae, *Anabaena azollae*, is considered to be the most promising because of the high productivity, good nutritive value and easiness of cultivation (Singh and Subudhi, 1978 and Van Hove and Lopez, 1982). Azolla is one of the plant resources with high biomass and protein production.

Azolla pinnata was used as a feedstuff in broiler chicken (Querubin *et al.*, 1986 and Parthasarathy *et al.*, 2002). However, there seems to be paucity of studies on the effect of dietary inclusion of Azolla on the production performance of the broilers.

Hence, a study was planned to evaluate the Azolla (*Azolla pinnata*) on production performance of commercial broiler chicken with the following objectives:

1. To study the effect of dietary inclusion of dried Azolla on production performance and biochemical changes of broiler chicken.
2. To study the effect of Azolla feeding on carcass characteristics and economics of feeding Azolla in broiler chicken.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Azolla is a small aquatic fern with multibranched stems, found in fresh water ecosystem of temperate and tropical regions of the world. In India, it is found in shallow fresh water lakes, ponds and ditches. It belongs to genus *Salviniaceae* and family *Azollaceae*. It is rich in protein and is a good source of other nutrients. Therefore, experiments are underway to find its suitability as an ingredient in livestock and poultry rations. Available literatures on the effect of dietary inclusion of Azolla and other aquatic plants on chicken and other species of poultry are reviewed in this chapter.

2.1 Occurrence and distribution of Azolla

According to Basak *et al.* (2002) there are six living species of Azolla in the world, of which four species viz., *A. filiculoides, A. caroliniana, A. mexicana and A. microphylla* are found in North and South America, Mexico, West Indies and British Columbia. The other species viz., *A. pinnata* widely occurs in tropical Africa, South East Asia, Japan and Australia, while *A. nilotica* has been reported to occur in Central Africa, Uganda, Sudan and Nambibia.

2.2 Nitrogen fixation

Azolla is important among the aquatic plants due to the occurrence of both *photosynthesis and nitrogen fixation in their leaves (Singh and Subudhi, 1978).* The nitrogen fixation takes place in the plant by the blue-green algae, *Anabaena azollae* present in the cavity of azolla, in symbiotic association. Due to rapid availability of fixed nitrogen to the frond, there is no additional requirement of nitrogen fertilizer for its growth.

Pillai *et al.* (2004) clarified that Azolla is a floating fern though it looks like algae. The fern Azolla, hosts a symbiotic blue green algae *Anabaena azollae* which is responsible for the fixation and assimilation of atmospheric nitrogen. Azolla in turn provides the carbon source and favourable environment for the growth and development of the blue green algae symbiont.

2.3 Meteorological observations

Somanathan (1980) reported a maximum temperature ranging from 31.14 to 35.14°C, minimum temperature ranging from 21.15 to 25.80°C and average relative humidity (R.H.) ranging from 58.49 to 84.39 per cent during the period from January to June in the years 1974 to 1978 at the Meteorological observatory unit, Mannuthy. The measurements were taken at latitude of $10^0 32''$ N, longitude of $76^0 16''$E and altitude of 22.25m above MSL for 5 years and average values were reported.

2.4 Nutritional value of Azolla

Buckingham *et al.* (1978) studied the proximate composition of *Azolla filiculoides* and reported that it contained 23.42 per cent total protein, 5.05 per cent ether extract (EE), 15.54 per cent ash, 26.58 per cent acid detergent fibre (ADF), 39.16 per cent neutral detergent fibre (NDF), 15.19 per cent cellulose and 9.27 per cent lignin. The composition of essential amino acids in the Azolla (g per 100 g protein) compared well with the reference protein sources like alfalfa, soybean and corn. They compared Azolla with corn and reported low methionine value (1.88 per cent) in Azolla with lysine content twice (6.45 per cent) as that in corn (2.76 per cent). Relative to alfalfa meal major minerals in Azolla were adequate and the calcium and phosphorus ratio was 2:1. Some of the trace minerals in Azolla were higher than in alfalfa; iron 4 times (1 mg/g), copper 3 times (28.05 μg/g) and manganese 22 times (771 μg/g).

Subudhi and Singh (1978) analyzed chemical composition of Azolla on dry matter basis. It contained 93.5% moisture, 24.0 to 30.0 per cent crude protein, 3.0 to 3.36 per cent crude fat, 0.4 to1.0 per cent calcium and 10.5 per cent ash. Azolla was found to be rich in vitamins, minerals (especially calcium 0.4 to 1.0 per cent and phosphorus 0.5 to 0.9 per cent), chlorophylls and carotenoids.

Querubin *et al.* (1986) studied chemical composition of three Azolla species viz., *A.caroliniana, A.microphylla and A.pinnata* and reported that nutrient content varied considerably. On dry matter basis, the values ranged between 17.59 and 23.69 per cent for crude protein; 1.93 and 2.93 per cent for ether extract; 13.19 and 16.54 per cent for crude fibre; 1.67 and 2.07 per cent for calcium and 0.46 and 0.77 per cent for total phosphorus. Azolla was comparable to ipil-ipil in gross energy, carotene and xanthophylls contents, but contain no mimosine. Species of Azolla were also rich in trace minerals.

Sanginga and Van Hove (1989) revealed that the mean protein content of Azolla (28.0 per cent) was comparable to that of some rich legumes like alfalfa. All Azolla strains contained a similar proportion of essential amino acids (55 per cent) mainly leucine, lysine, arginine and phenyl alanine + tyrosine to non-essential amino acids (45 per cent). Whereas, sulfur containing amino acids like methionine and cysteine were present in smaller amounts.

Becerra *et al.* (1990) reported that water plants such as *Azolla filiculoides* were highly productive source of protein rich biomass and are an ideal complement for the low fibre tropical feed resources being developed as alternatives to cereals in poultry and pig diets. It contained 3.8 per cent nitrogen, 1.7 per cent calcium, 0.7 per cent phosphorus and 1.8 per cent potassium.

Tamang and Samanta (1993) observed that *Azolla pinnata* contained 7.0 per cent dry matter, 15.40 per cent crude protein, 14.10 per cent crude fibre, 2.80 per cent ether extract, 20.40 per cent total ash, 47.40 per cent NFE, 51.90 per cent acid detergent fibre (ADF), 67.50 per cent neutral detergent fibre (NDF), 1.50 per cent calcium and 0.40 per cent phosphorus on dry matter basis.

Azolla contained 26.70 per cent crude protein, 11.0 per cent crude fibre, 4.60 per cent ether extract, 0.8 per cent calcium and 0.4 per cent total phosphorus (Becerra *et al.,* 1995b). Azolla was also found to be rich in vitamins, minerals, chlorophylls and carotene (326 mg/kg).

Ali and Leeson (1995) observed that *Azolla pinnata* contained 9.2 per cent dry matter and 16.5 per cent crude protein, 12.5 per cent crude fibre, 1.6 per cent fat, 36.1 per cent total ash, 47.8 per cent NFE, 46.7 per cent ADF, 1.4 per cent calcium, 0.31 per cent phosphorus and 4.38 MJ/kg apparent metabolisable energy value on dry matter basis. In comparison with alfalfa meal (314.6 g/kg DM), Azolla had less crude fibre and similar crude protein value. The amino acid values of Azolla seem comparable with other aquatic plants and alfalfa meal of similar protein level. Amino acid composition of Azolla revealed that it contained 1.5 per cent methionine, 0.9 per cent cystine, 3.8 per cent lysine, 5 per cent arginine, 0.5 per cent tryptophan, 3 per cent tyrosine, 4 per cent threonine, 4 per cent serine, 4.6 per cent phenyl alanine, 8.3 per cent aspartic acid, 9.6 per cent glutamic acid, 4 per cent proline, 5.2 per cent glycine, 5.8 per cent alanine, 5.1 per cent valine, 4.2 per cent isoleucine, 7.7 per cent leucine and 1.6 per cent histidine. These amino acid values of Azolla seem comparable with those found in other aquatic plants and alfalfa meal of similar protein level.

Sarria and Preston (1995) compared amino acid balance in Azolla and soybean with the ideal protein (expressed as per cent of lysine =100) and reported superior balance of amino acids in Azolla compared with soybean, with values of lysine (100 per cent and 100 per cent), methionine + cystine (63 per cent and 48 per cent), threonine (72 per cent and 61 per cent), tryptophan (31 per cent and 22 per cent), valine (103 per cent and 83 per cent), isoleucine (83 per cent and 90 per cent), leucine (139 per cent and 133 per cent) and phenyl alanine + tyrosine (149 per cent and 139 per cent) in Azolla and soybean, respectively.

Ardakani *et al.* (1996) analyzed chemical composition of Azolla and reported that it contained 17.67 per cent crude protein, 21.5 per cent crude fibre, 2.49 per cent ether extract, 3.2 per cent calcium, 0.17 per cent phosphorus and 3949 kcal/kg gross energy on dry matter basis.

Khatun *et al.* (1999) studied the proximate composition of dried *Azolla pinnata* and stated that it contained 905.8 g/kg dry matter. The crude protein content (285.4 g/kg) was similar to seasame meal. They also reported that *Azolla pinnata* contained 219.8 g/kg digestible protein, 123.8 g/kg crude fibre and 7.59 MJ/kg metabolisable energy.

Parthasarathy *et al.* (2001b) studied the chemical composition of various species of Azolla and opined that it could be used as an unconventional feed source for broilers. The content of crude protein, ether extract, crude fibre, NFE and total ash ranged from 24.91 to 27.22 per cent, 2.52 to 3.01 per cent, 13.84 to 16.40 per cent, 38.85 to 44.06 per cent and 12.8 to 16.26 per cent, respectively. The apparent and true metabolisable energy of *A.pinnata* were 1529 and 1855 kcal/kg DM respectively. The mineral profile of various species indicated that *Azolla microphylla* contained higher levels of calcium (2.11 per cent) and phosphorus (1.08 per cent) compared to other species of Azolla.

Parthasarathy *et al.* (2002) evaluated the chemical composition of *Azolla pinnata* and indicated that it was a fairly rich source of crude protein (26.02 per cent) and total ash (12.3 per cent). The other proximate principles present were 13.60 per cent crude fibre, 2.37 per cent EE and 45.71 per cent NFE. The macro minerals like calcium and phosphorus were 1.24 per cent and 0.72 per cent, respectively and more or less in the ratio of 2:1.

Basak *et al.* (2002) analysed the chemical composition of *Azolla pinnata* and revealed that the meal contained 25.78 per cent crude protein, 15.71 per cent crude fibre, 3.4 per cent ether extract, 15.76 per cent ash and 30.08 per cent NFE on dry matter basis.

Pillai *et al.* (2004) reported that Azolla is very rich in protein and almost all essential amino acids, vitamin A precursor carotene, several growth promoter intermediaries and minerals like calcium, phosphorus, potassium, iron, magnesium etc. The protein content was 25 to 35 per cent (on dry matter basis), mineral content 10 to15 per cent, amino acids and bioactive substances and bio-polymers constitute 7 to10 per cent. Carbohydrate and oil contents in Azolla were very low.

Alalade and Iyayi (2006) conducted studies to evaluate the potential of Azolla meal. Chemical analysis indicated that it contained 21.4 per cent crude protein, 12.7 per cent crude fibre, 2.7 per cent ether extract, 16.2 per cent ash and 47.0 per cent carbohydrate on dry matter basis. The gross energy value of *Azolla pinnata* was

2039 kcal/kg. The concentrations of calcium, phosphorus, potassium and magnesium were 1.16, 1.29, 1.25 and 0.25 per cent respectively. Lysine, leucine, arginine and valine were the predominant amino acids found in Azolla meal, while tryptophan and other sulphur containing amino acids were deficient.

Raseena (2006) analysed the chemical composition of *Azolla pinnata* on dry matter basis and revealed that it contained 5.25 per cent dry matter, 23.14 per cent crude protein, 12.20 per cent crude fibre, 3.50 per cent ether extract, 17.50 per cent ash, 43.66 per cent NFE, 2.29 per cent calcium and 0.39 per cent phosphorus.

2.4.1 Nutritive Value of Other Aquatic Plants

Muztar *et al.* (1976) reported that some aquatic plants like Hydrilla (*Hydrilla verticililata*) and water hyacinth (*Eichhornia crassipes*) were variable in crude protein, ranges from 5.6 to 15.5 per cent, and contained somewhat less crude fibre and ether extract than dehydrated alfalfa meal. They were high in ash (18.0 to 62.5 per cent) and xanthophylls content than conventional forage crops.

Lipstein and Hurtwitz (1980) analysed the algae chlorella and reported to contain 89 g/kg moisture and 574 g/kg protein, 69.4 g/kg total lipids, 22.0 g/kg fiber, 2.0 g/kg calcium, 11.7 g/kg phosphorus and 11.6 MJ/kg metabolisable energy on dry matter basis. The algae lipids contained more linolenic (18:3) and less linoleic (18:2) acids than are usually found in maize and soybean oils. It also had a high pigment concentration of xanthophylls and carotene which ranged from 650 to 1100 mg/kg and from 120 to 260 mg/kg, respectively.

Viswanathan and Thirumalai (1987) studied the chemical composition of seaweed and reported that it contained 4.94 to 17.10 per cent crude protein, 1.09 to 7.08 per cent calcium, 0.01 to 0.3 per cent iodine on dry matter basis.

Ross and Dominy (1990) analysed chemical composition of spirulina on dry matter basis. It contained 94.5 per cent DM, 60.5 per cent protein, 0.95 per cent ether extract, 6.9 per cent ash; 0.28 per cent calcium, 0.95 per cent phosphorus, 0.47 per cent sodium and 0.99 per cent potassium. But spirulina had much lower cystine and lysine content than soyabean meal.

Becerra *et al.* (1995a) reported that duckweed contained 4.5 per cent dry matter and 26.3 per cent protein, 3.2 per cent ether extract, 11.0 per cent crude fibre, 15.9 per cent ash, 1.1 per cent calcium, 0.5 per cent phosphorus and 535 mg/kg carotene on dry matter basis.

Men *et al.* (1995) found that duckweed contained 4.7 per cent dry matter, 38.6 per cent protein, 9.82 per cent ether extract, 18.7 per cent crude fibre, 8.58 per cent nitrogen free extract, 19.0 per cent ash, 0.71 per cent calcium, 0.62 per cent phosphorus, 4.9 per cent potassium, 0.14 per cent sodium, 0.27 mg/kg iron, 1723 mg/kg manganese, 75 mg/kg zinc, 20 mg/kg copper and 1025 mg/kg carotene.

Islam (2002) estimated the chemical composition of duckweed. On dry matter basis it contained 7.0 to 40.0 per cent crude protein. The essential amino acid profile of duckweed is similar to other animal proteins with the exception of methionine. Dried duckweed could be used in poultry diets to partially replace fish meal, soybean meal and alfalfa meal. It was concluded that duckweed could be included as a protein concentrate mixture for layers and broilers.

Khang and Ogle (2004a) analysed nutrient composition of duckweed. It contained 6.0 to 7.0 per cent dry matter and 37.0 per cent crude protein, 6.7 per cent ether extract, 7.7 per cent crude fibre, 1.3 per cent calcium, 0.9 per cent phosphorus, 1.6 per cent lysine and 1.0 per cent methionine on dry matter basis.

Khanum *et al.* (2005) reported that the duckweed contained 7.03 per cent dry matter and 39.0 per cent protein, 7.7 per cent ether extract, 12.3 per cent crude fibre, 14.7 per cent ash and 85.3 per cent organic matter on dry matter basis.

Khandaker *et al.* (2007) analysed nutrient composition of duckweed. It contained 88.07 per cent dry matter and 29.20 per cent crude protein, 5.06 per cent ether extract, 10.48 per cent crude fibre, 27.0 per cent nitrogen free extract, 2.39 per cent calcium, 0.71 per cent phosphorus, 4.7 per cent lysine and 1.4 per cent methionine on dry matter basis..

2.5 Effect of Azolla on body weight

Subudhi and Singh (1978) studied the effect of *Azolla pinnata* in White Leghorn female chicks at the levels of 16.0, 12.5 and 5.0 per cent fresh Azolla on dry weight basis along with 50, 75 and 100 per cent intake of control diet, respectively from 7 to 14 weeks of age. They reported that the growth in the treatment of 75 per cent feed with 12.5 per cent Azolla was almost comparable (518 g) to the control birds (494 g). The birds receiving normal diet with 5 per cent Azolla grew faster (614 g) than the control. It was concluded that 20 to 25 per cent of commercial feed could be replaced by supplementing fresh Azolla in the diet.

Querubin *et al.* (1986) studied the feeding value of Azolla meal in broiler chicks at the levels of 0, 5, 10 and 15 per cent on dry matter basis. Average body weight gain of the birds was not significantly affected with Azolla meal in the diet. The results of this study indicated that Azolla meal inclusion in broiler ration was best at 10 per cent although the growth rate of birds was not affected up to 15 per cent level.

There were no significant differences in growth rate (24.6, 29.4, 34.0 g/day) in broiler chicks fed with 0, 10 and 15 per cent replacement of the protein from Soybean meal with *Azolla filiculoides* (Sarria and Preston, 1995).

Ali and Leeson (1995) studied the effect of dietary inclusion of Azolla (100 g/kg based on digestible protein) and snail meal (80 g/kg based on digestible protein) in broiler chicken separately for 14 days. The weight gain was similar (236 and 242 g, respectively) to that found in control birds (226.3 g) fed maize-soybean meal diet.

Becerra *et al.* (1995b) conducted two feeding trials to determine the effect of feeding *Azolla microphylla* as partial replacement of the protein in boiled soyabean in diets based on sugar cane juice for meat ducks. In first trial, daily weight gains (g) were 28.6, 30.4, 28.3, 24.1 and 21.4 for the control and 15, 30, 45 and 60 per cent Azolla inclusion levels, respectively. The gain decreased with increasing level of Azolla when it exceed 15 per cent of the dietary protein. In second trial, daily gains (g) were 32.6, 31.5, 30.2, 28.2 and 24.1 for the respective treatments with decrease in gain with increasing Azolla level.

Ardakani *et al.* (1996) observed best performance in weight gain for birds fed with 6 per cent fresh Azolla.

Parthasarathy *et al.* (2001a) indicated that broiler birds receiving 5 per cent Azolla from 0 to 5 weeks of age had similar weight gain as that of control (1106 vs 1083 g), while the birds on 10, 15 and 20 per cent Azolla diets had significantly poor weight gain.

Basak *et al.* (2002) reported significant (P<0.01) improvement in the live weight (1637 g) of broilers when they were fed with 5 per cent Azolla meal by replacing sesame meal than control diet fed birds (1579 g) at 6 weeks of age. They also observed that use of higher levels of Azolla meal (10 and 15 per cent) resulted in poor growth rate than control fed groups.

Parthasarathy *et al.* (2002) reported that broiler birds on basal and 5 per cent Azolla diets replacing 2.6 per cent wheat bran and 2.4 per cent fish meal gained more or less similar body weights (1803 and 1816 g respectively) at 8 weeks of age. However, higher levels of 10 and 20 per cent Azolla resulted in significant reduction in live weight gain (1752 and 1650 g, respectively).

Pillai *et al.* (2004) carried out feeding trials in poultry with different combinations of commercial feed and Azolla and reported that 20 to 25 per cent of commercial feed could be replaced by supplementing it with fresh Azolla in the diet. Birds fed with

75 per cent of the regular feed with 12.5 per cent Azolla weighed almost equal to the birds with 100 per cent feed. The birds receiving normal diet with 5 per cent extra Azolla grew faster than the bird with 100 per cent feed alone and there was 10 to12 per cent increase in the total body weight.

Alalade and Iyayi (2006) observed no significant difference in weight gain (95.43, 95.22, 98.62 and 93.44 g) of chicks fed with Azolla meal at the levels of 0, 5, 10 and 15 per cent, respectively. But the group fed diet containing 10 per cent Azolla meal performed non statistically better than the control diet fed birds.

Raseena (2006) reported that the mean twenty sixth week body weight of layer quails fed with Azolla at 0, 1.5, 3.0 and 4.5 per cent levels from six to twenty six weeks of age were 222.27, 223.31, 220.52 and 223.30 g, respectively. The mean body weight gain during the experimental period (6 to 26 weeks of age) was 37.74, 37.27, 35.51 and 36.74 g, respectively. The statistical interpretation of the results showed no significant difference in both body weight and weight gain among different dietary treatment groups.

Alalade *et al.* (2007) studied the performance of growing pullets fed diets containing Azolla meal at 0, 5, 10 and 15 per cent levels. There were no significant differences in weight gain (95, 102, 92 and 91 g/bird/week respectively). But birds on 5 per cent Azolla meal gained non-statistically better than those on the control diet.

2.5.1 Effect of Other Aquatic Plants on Body Weight

In an experiment, Muztar *et al.* (1976) included dehydrated alfalfa meal and each of the dried aquatic plant meal (cladophora, duckweed, pond weed, milfoil and vallisneria) in place of corn and soybean meal of control diet in egg strain male chicks at the levels of 2.5, 5 and 10 per cent so as to maintain all diets equinitrogenous. Diets containing any level of dehydrated alfalfa or aquatic plants gave results, which were significantly superior to that of control diet.

Lipstein and Hurtwitz (1980) included 150 g chlorella algae meal per kg in the broiler starter and finisher diets and observed a reduction in weight gain by 5 and 10 per cent, respectively compared with the control diet.

Lizama *et al.* (1988) reported that body weight gain in chicks fed with 5 and 10 per cent Elodea by replacing yellow maize and soybean meal were similar (4.41 and 4.22 g/bird/day) compared to controls (4.45 g/bird/day), but 5 per cent Hydrilla significantly ($P<0.05$) depressed weight gain (3.6 g/bird/day).

One day old male broiler chicks were fed experimental diets containing 0, 1.5, 3.0, 6.0 or 12.0 per cent of spirulina for 41 days (Ross and Dominy, 1990). Although the growth of chicks fed the spirulina diets was not significantly different from that of the chicks receiving control diet, the birds receiving the 12 per cent spirulina diet grew slower than the chicks fed all of the other spirulina diets.

Hausteina *et al.* (1992) evaluated the growth of broiler chickens on diets containing various levels of *Lemna gibba.* Groups of broiler chicks were fed on diets containing 0 to 400 g *Lemna gibba* per kg for 3 weeks. The birds were then changed to standard diets for further 2 weeks. As the level of *Lemna gibba* increased, feed consumption and weight gain decreased. However, when diets were changed to the standard diet, compensatory growth was observed.

Benicio *et al.* (1993) reported that weight gain of broiler chicken during the starting period was not affected by inclusion of 3 per cent water hyacinth in pelleted diets. However, performance was affected by larger amounts.

Haustein *et al.* (1994) observed that weight gain of broiler chicks fed with 15 per cent dried duckweed was similar to that of controls. Increase in duckweed above 15 per cent resulted in significant decrease in weight gain. Females given diets containing 5 per cent duckweed had increased final weight.

Becerra *et al.* (1995a) studied the effect of partial replacement of soybean with duckweed (0, 15, 25, 35 and 45 per cent) in sugar cane juice based diets and reported significantly (P<0.01) reduced weight gain (29.5, 25.5, 24.4, 21.6 and 20.8 g, respectively) in cherry valley growing ducks.

Men *et al.* (1995) reported that the rate of live weight gain was significantly higher for cross bred meat type ducks when fed with diets containing 30 and 45 per cent duckweed than control diet. The diet with complete replacement of the soya beans (100 per cent) with duckweed had slightly better growth than on the control diet.

Men *et al.* (1996) included duckweed in Muscovy duck diets replacing soybean meal. The basal diet was broken rice and the protein supplement came from roasted soybean to supply 100 (control), 40 (DW60), and zero (DW100) per cent protein on three treatments. They observed that live weight at 70 days and growth rate from 28 to 70 days were highest for Muscovy males fed the control ration which contained broken rice balanced with protein from roasted soybeans to supply 100 per cent requirement and duckweed *ad libitum*. There was no difference in daily weight gain between DW60 and DW100 diets for both males and females (ranged from 21.7 to 22.5 g for females and 27.6 to 28.3 g for males).

Toyomizu *et al.* (2001) studied the effect of dietary spirulina on growth performance of 21 days old male growing broiler chickens. The chicks were fed experimental diets containing spirulina at 0, 40, or 80 g/kg for 16 days. There were no significant differences among treatments in body weights.

Islam *et al.* (1996) reported that the live weight gain was lower with starter and grower test diets containing 13.6 and 5.9 per cent leaf protein concentrate of water hyacinth on White Leghorn chicks.

Islam *et al.* (1997) measured all growth parameters, which had a linear declining trend as the proportion of duckweed increased in the diet replacing complete fish meal by duckweed and soybean meal in broilers.

Islam (2002) observed that dehydrated duckweed, when substituted for dried alfalfa meal up to 5 per cent of mixed poultry feeds, produced superior weight gain in chicks up to 3 weeks of age.

In a study, Khang and Ogle (2004a) found that the one day old local breed chicks fed with duckweed had somewhat higher weight gain (8.3 g/day) compared with chicks fed the diets without duckweed (7.8 g/day).

Khanum *et al.* (2005) conducted experiment to determine the intake and digestibility of fresh duckweed in growing ducks. They fed ducks with three diets, viz., control diet, 50 per cent control diet + *ad libitum* fresh and harvested duckweed (DWH) and 50 per cent control diet + ducks were allowed to graze on duckweed in lagoon (DWG). They observed that average daily body weight gain (6.53 and 6.28 g, respectively) and the final live weight (1256 and 1189 g, respectively) were not different between duckweed diets fed ducks, but significantly ($P<0.01$) lower than that in the control diet (9.09 and 1345 g).

Khandaker *et al.* (2007) studied the effect of feeding duckweed that replaced conventional protein supplement mustard oil cake (MOC) in the diets for laying ducks. The diets were based on rice by products, where soybean meal and mustard oil cake as protein source. The control diet A contained 15 per cent MOC and 5, 10 and 15 per cent MOC was replaced with dry duckweeds in the diets B, C and D respectively. The body weights of ducks at the beginning of the experiment were almost similar (1428 to 1450 g). The body weight of ducks in all four treatment groups increased in first fortnight and thereafter it was almost static. In fifth fortnight, the body weights were between 1478 and 1496 g.

2.6 Effect of Azolla on feed intake

Querubin *et al.* (1986) studied the feeding value of Azolla meal in broiler chicks at the levels of 0, 5, 10 and 15 per cent on dry matter basis. Average feed consumption of the birds increased significantly at 5 per cent level. They also found that feed intake consistently increased with higher levels of Azolla meal in the diet.

Sarria and Preston (1995) fed broiler chicks with ration containing 0, 10 and 15 per cent replacement of the protein from soybean meal with *Azolla filiculoides* and observed no significant difference in feed intake (59.0, 58.3 and 67.0 g, respectively).

Ali and Leeson (1995) studied the effect of dietary inclusion of Azolla (100 g/kg on digestible protein basis) and snail meal (80 g/kg on digestible protein basis) in broiler chicken separately for 14 days and found that the feed consumptions were similar (318.8 g for Azolla and 331.2 g for snail meal) to that of control birds fed maize soybean diet (321.8 g).

Khatun *et al.* (1999) reported that feed consumption of laying hens fed with diets containing 150 and 200 g/kg Azolla meal on a digestible protein and digestible amino acid basis were similar (108.2 and 107.3 g/bird/day, respectively) to that of birds fed with control diet (107.3 g/bird/day). But feed consumption significantly increased (112.6 and 113.6 g/bird/day, respectively) with 150 and 200 g/kg Azolla meal on a total protein and total amino acid basis.

Parthasarathy *et al.* (2001a) observed that feed intake decreased as the level of Azolla was increased from 0 to 15 per cent in broiler birds, but there was a slight increase in feed intake at 20 per cent level during starter period.

Basak *et al.* (2002) found that mean cumulative feed consumption was almost similar (3140.0, 3104.99, 3133.33 and 3149.99 g/bird) in different dietary treatments namely 0, 5, 10 and 15 per cent Azolla by replacing sesame meal from seven to forty two days of age and the differences were non significant at all ages of experimental period in broiler chicks. They concluded that inclusion of Azolla in broiler diet did not affect the feed consumption up to 15 per cent.

Alalade and Iyayi (2006) observed that 10 and 15 per cent Azolla meal in the diet of chicks significantly reduced the average weekly feed intake (231.28 and 224.38 g/week, respectively). But average weekly feed intake was similar in 5 per cent Azolla meal inclusion in diets (270.73 g/week) to that of control diet (286.95 g/week).

Raseena (2006) reported that the cumulative mean daily feed intake of Japanese quails from seven to twenty six weeks of age was 28.35, 29.96, 30.05 and 29.70 g for different dietary groups fed with Azolla at 0, 1.5, 3 and 4.5 per cent respectively. The differences were statistically non significant.

Alalade *et al.* (2007) studied the performance of growing pullets fed diets containing Azolla meal at 0, 5, 10 and 15 per cent levels. There were no significant differences in feed intake (731, 708, 653 and 659 g/bird/week respectively).

2.6.1 Effect of Other Aquatic Plants on Feed Intake

Lipstein *et al.* (1980) observed no significant effect of dietary algae meal chlorella on feed intake of layer birds.

Lizama *et al.* (1988) reported that broiler chicks fed with 5 and 10 per cent dried ground Elodea had similar feed intake (7.28 and 7.11 g/bird/day, respectively) to that of control chicks (7.13 g/bird/day).

Ross and Dominy (1990) used layer Japanese quail to study the effect of 0, 1.5, 3.0, 6.0 and 12.0 per cent of spirulina in their diet. Feed consumed per bird per day was 24.9, 23.3, 24.0, 24.1 and 24.8 g, respectively. The group fed the diet with 1.5 per cent spirulina consumed less feed ($p<0.05$) compared with other groups.

Hausteina *et al.* (1992) studied the effect of *Lemna gibba* in broiler chicken. The chicks were fed with diets containing 0 – 400 g /kg *Lemna gibba* for 3 weeks. The birds were then changed to standard diets for further 2 weeks. As the level of Lemna gibba increased, decreased feed consumption was noticed.

Benicio *et al.* (1993) included 3 per cent water hyacinth in pelleted diets for broiler chickens during the starting period and found that feed intake was not affected by inclusion of water hyacinth. However, performance was affected by inclusion of larger amounts.

Becerra *et al.* (1995a) reported that daily intake of dry matter and crude protein reduced significantly with decreasing soybean supply in growing ducks by increasing duckweed inclusion in the diet.

Men *et al.* (1996) included duckweed in Muscovy duck diets replacing soybean meal. The basal diet was broken rice and the protein supplement came from roasted soybean to supply 100 (control), 40 (DW60), and zero (DW100) per cent protein on three treatments. They observed that the intakes of duckweed increased as a percentage of liveweight in DW 100 or diets without soyabeans.

Islam *et al.* (1997) studied the effect of complete replacement of dietary fish meal (FM) by duckweed (DW) and soybean meal (SBM) on the performance of broilers. They formulated four different diets. Diet A contained control with 12 per cent FM, diets B contained 3 per cent DW+13.5 per cent SBM, diet C contained 6 per cent DW+11.5 per cent SBM and diet D contained 9 per cent DW+10 per cent SBM. The results showed that all FM protein of control diet replaced by DW and SBM had depressed feed intake (3.563, 3.100, 3.162 and 2.700 kg/bird, respectively).

Khang and Ogle (2004a) studied the effect of dietary protein level and a duckweed supplement on the growth rate of one day old chicks fed with 18, 20, and 22 per cent of crude protein on DM basis, with or without fresh duckweed *ad libitum*. Total daily dry matter intakes were slightly higher in the 20 and 22 per cent crude protein diets (12.0g and 11.7 g, respectively) compared with that of 18 per cent diet (11.1 g). When duckweed was supplied with 20 and 22 per cent of crude protein, the feed intake was higher, but the differences were not significant between treatments.

2.7 Effect of Azolla on feed conversion ratio (FCR)

Querubin *et al.* (1986) studied the feeding value of Azolla meal in broiler chicks at levels of 0, 5, 10 and 15 per cent on dry matter basis and reported that the feed efficiency decreased significantly only at 15 per cent Azolla meal inclusion in the diet.

Becerra *et al.* (1995b) conducted two feeding trials to determine the effect of feeding *Azolla microphylla* as partial replacement of the protein in boiled soya bean in diets based on sugar cane juice for meat ducks. In first trial, feed conversion ratios decreased with increasing consumption of Azolla from 15 to 60 per cent in diets. In second trial feed conversion ratios increased with increasing consumption of Azolla from 15 to 45 per cent in the diet.

Ardakani *et al.* (1996) reported better FCR in broiler birds fed with 6 per cent fresh Azolla compared with control fed birds.

Khatun *et al.* (1999) found that feed efficiency improved in laying hens when they were fed with Azolla diets formulated on a digestible nutrient basis.

Parthasarathy *et al.* (2001a) reported that the broiler birds fed with control diet had feed efficiency of 2.13, while the birds receiving 5 per cent Azolla had improved feed efficiency (2.03). However, poor feed efficiencies were observed in 10 per cent (2.34), 15 per cent (2.46) and 20 per cent (2.57) Azolla fed groups.

Basak *et al.* (2002) studied the feasibility of inclusion of Azolla in broiler ration and reported that FCR improved significantly at 5 per cent level of inclusion. The FCR obtained by 5 per cent Azolla feeding and control group without Azolla were 2.07 and 2.17 respectively during 2 to 6 weeks of age. They also observed significantly decreased FCR at 10 and 15 per cent Azolla meal (2.38 and 2.50) in the diet of broiler chicken.

Parthasarathy *et al.* (2002) obtained feed and protein efficiency ratio which were in the same order as in basal and 5 per cent Azolla diets replacing 2.6 per cent wheat bran and 2.4 per cent fish meal, where as these were significantly decreased as the level of Azolla increased from 10 to 20 per cent.

Alalade and Iyayi (2006) observed that feed conversion ratio decreased from 3.13 in control diet fed chicks to 2.54 in birds fed 10 per cent Azolla and 2.55 in 15 per cent Azolla fed group.

Raseena (2006) reported that the cumulative mean feed conversion ratio in layer quails based on per dozen eggs for the different dietary groups fed with 0, 1.5, 3 and 4.5 per cent Azolla were 0.41, 0.42, 0.45 and 0.43, respectively. The corresponding values calculated based on per kg egg mass were 3.02, 3.11, 3.23 and 3.12, respectively. The cumulative mean FCR was numerically inferior for 3.0 per cent Azolla fed group followed by 4.5 per cent, 1.5 per cent and control fed groups. Statistical analysis revealed no significant difference among the treatments.

Alalade *et al.* (2007) studied the performance of growing pullets fed Azolla meal in diets containing 0, 5, 10 and 15 per cent levels and reported that FCR differ significantly (10.54, 9.33, 10.63 and 8.38, respectively) in different dietary treatment groups.

2.7.1 Effect of Other Aquatic Plants on Feed Conversion Ratio (FCR)

Muztar *et al.* (1976) observed that feed efficiency decreased as the level of dehydrated alfalfa or dried aquatic plant was increased in the diet of male egg strain chicken.

Lipstein *et al.* (1980) observed no significant effect of dietary algae meal, chlorella on feed conversion of layer birds.

Lizama *et al.* (1988) reported that 5 and 10 per cent Elodea fed broiler chicks replacing yellow maize and soybean meal had similar FCR (1.65 and 1.69 respectively) as that of controls (1.61).

Ross and Dominy (1990) conducted an experiment in which one day old male broiler chicks were fed with diets containing 0, 1.5, 3.0, 6.0 or 12.0 per cent of spirulina for 41 days. There was no significant difference in feed utilization efficiency (1.78, 1.78, 1.80, 1.78 and 1.77 g/g, respectively) among the treatments.

Hausteina *et al.* (1992) studied the effect of *Lemna gibba* in broiler chicks by incorporating it in diets at the levels of 0 to 300 g for 4 weeks period. After 4 weeks period this group was divided into two subgroups. One of these sub groups was maintained on the *Lemna gibba* diets, while other sub group changed to standard diet. Birds fed on standard diet had lower feed conversion ratio than those maintained on *Lemna gibba* diets.

Benicio *et al.* (1993) included 3 per cent water hyacinth in pelleted diets for broiler chickens during the starting period and found that feed efficiency was not affected by the inclusion of water hyacinth. However, performance was affected by inclusion of larger amounts.

Becerra *et al.* (1995a) studied the effect of partial replacement of soybean with duckweed (0, 15, 25, 35 and 45 per cent) in sugar cane juice based diets and reported that FCR was poorer (4.9, 6.0, 6.1, 6.7 and 6.6, respectively) with decreasing soya bean supply in cherry valley growing ducks.

Men *et al.* (1995) studied the effect of feeding duckweed (DW) replacing the roasted soybeans in diets based on broken rice for cross bred meat ducks. The five diets were fed *ad libitum* supplemented with 27 g/day roasted soybeans (control, DW zero), 19g/day soybean (DW 30 per cent), 15 g/day soybean (DW 45 per cent), 12g/day soybean (DW 60 per cent) and zero soybean (DW 100 per cent). They observed that FCR was best in ducks fed with control diet (3.7, 4.2, 4.2, 4.1, and 4.2 respectively). However, it did not differ significantly among the diets containing duckweed.

Men *et al.* (1996) included duckweed in Muscovy duck diets replacing soybean meal. The basal diet was broken rice and the protein supplement came form roasted soybean to supply 100 (control), 40 (DW 60), and zero (DW 100) per cent protein on three treatments. They reported that the feed dry matter conversion was poorer (4.06 to 4.17 for females and 4.12 to 4.23 for males) on all duckweed diets compared with that of control diet (3.76 and 3.24 for females and males, respectively).

Islam *et al.* (1997) reported that the complete replacement of dietary fish meal by duckweed and soybean meal depressed FCR in broilers from 2.45 to 2.50.

Islam (2002) opined that inclusion of 15 per cent Lemna and 15 per cent Wolffia in the diets of layers produced no significant difference in feed conversion when compared with those of control groups.

Khang and Ogle (2004b) got highest value for FCR, when hens were fed with fresh duckweed replacing 75 per cent of the protein from roasted soybeans in a diet based on broken rice.

Khandaker *et al.* (2007) studied the effect of feeding duckweed that replaced conventional protein supplement mustard oil cake (MOC) in the diets for laying ducks. The diets were based on rice by products with soybean meal and mustard oil cake as protein source. The control diet A contained 15 per cent MOC and 5, 10 and 15 per cent MOC was replaced with dry duckweeds in the diets B, C and D respectively. For the production of 1 kg egg, it required 3.98, 4.18, 4.50 and 4.65 kg feed for the ducks of treatment groups A, B, C and D, respectively. Feed conversion efficiency was superior in ducks fed standard diet and inferior in diet where 15 per cent MOC was replaced by duckweed. It was observed that FCR decreased with the inclusion of increased proportion duckweed in place of MOC in the diets.

2.8 Effect of Azolla on carcass characteristics

Becerra *et al.* (1995a) conducted feeding trials to determine the effect of *Azolla microphylla* as partial replacement of the protein in boiled soyabeans in diets based on sugar cane juice for meat ducks. Carcass studies indicated that there were no significant differences in carcass percentage and weight of heart, liver and gizzard compared with control when broilers received 15, 30, 45 and 60 per cent Azolla replacing soyabeans.

Basak *et al.* (2002) reported that dressing per cent significantly increased on diet with 5 per cent Azolla meal (72.16 per cent) replacing sesame meal in broiler diets, compared with control (69.38 per cent). However, the dressing per cent decreased with 10 per cent (68.24 per cent) and 15 per cent (68.78 per cent) replacements. Giblet per cent was significantly higher in 15 per cent Azolla meal fed groups followed by 10 and 5 per cent and control fed groups

Parthasarathy *et al.* (2002) reported that broiler birds fed with 5 per cent Azolla diet had significantly ($P<0.05$) higher dressing percentage (69.66 per cent) than control (67.79 per cent) at 8 weeks of age.

Raseena (2006) found that the mean per cent giblet, ovary and oviduct weights were numerically higher for Azolla supplemented groups (1.5, 3.0 and 4.5 per cent) compared to those of control birds fed standard quail layer diet. The group fed with 1.5 per cent Azolla recorded numerically highest dressed yield (83.73 per cent) and the group fed with 4.5 per cent Azolla recorded numerically highest ready-to-cook-yield (60.07). While the statistical analysis of the mean values of these traits showed no significant difference.

2.8.1 Effect of Other Aquatic Plants on Carcass Characteristics

Becerra *et al.* (1995a) studied the effect of partial replacement of soybean with duckweed (0, 15, 25, 35 and 45 per cent) in sugar cane juice based diets and reported that carcass yield was not affected by dietary treatment (73, 69, 61, 68 and 70 per cent, respectively). The yield of all edible parts did not differ among the treatments.

Men *et al.* (1995) studied the effect of feeding duckweed (DW) replacing the roasted soybeans in diets based on broken rice for cross bred meat ducks. The five diets were supplemented with 27 g/day roasted soybeans (control, DW zero), 19g/day soybean (DW 30 per cent), 15 g/day soybean (DW 45 per cent), 12g/day soybean (DW 60 per cent) and zero soybean (DW 100 per cent). There were no significant differences in carcass yield and giblet weight in birds fed with different dietary groups.

Men *et al.* (1996) included duckweed in Muscovy duck diets replacing soybean meal. The basal diet was broken rice and the protein supplement came form roasted soybean to supply 100 (control), 40 (DW 60), and zero (DW 100) per cent protein on three treatments. Carcass yield was reduced on the duckweed treatments but differences were small.

Toyomizu *et al.* (2001) studied the effect of dietary spirulina on growth performance of 21 day old broiler chickens fed with diet containing spirulina at 0, 40, or 80 g/kg for 16 days. No significant differences were observed among treatments in carcass yields and giblet weights.

Khanum *et al.* (2005) fed ducks with three diets (control diet, 50 per cent control diet + *ad libitum* fresh and harvested duckweed (DWH), 50 per cent control diet + ducks were allowed to graze on duckweed in lagoon (DWG)). The treatment effect on carcass characteristics did not vary significantly (P>0.05), except for a significant (P<0.05) lower live weight (1310g) prior to slaughter in DWH. Carcass yields were 70.7, 70.2 and 64.7 per cent, respectively.

2.9 Effect on serum profiles

2.9.1 Serum cholesterol

Reddy *et al.* (2004) evaluated antioxidant and hypolipidemic effects of spirulina and natural carotenoids in broiler chicken. They found that spirulina at 1 per cent level with natural carotenoids at 10 mg/kg in diet resulted in decreased levels of total serum lipid and cholesterol (412.36 and 223.67 mg/dl, respectively) than control diet (543.07 and 304.43 mg/dl, respectively).

Shim *et al.* (2004) reported that broiler chicks fed with either 0.25 or 0.5 per cent dietary *Codonopsis lanceolata* root showed decreased serum triglyceride, total cholesterol and low density lipoprotein cholesterol levels of 41.9, 142 and 67.2 mg/dl, respectively for 0.25 per cent and 38.5, 143 and 64.6 mg/dl, respectively for 0.5 per cent groups than control fed group (47.7, 162 and 87.3 mg/dl, respectively).

Lonkar (2006) studied the effect of garlic powder and neem seed cake on cholesterol content in broiler chicken. Supplementation of garlic powder at 1 per cent (GP), neem seed cake (NSC) at 2 per cent and a combination of 0.5 per cent GP and 1 per cent NSC and 1 per cent GP and 2 per cent NSC resulted in a significant reduction in serum cholesterol level. The extent of reduction in 0.5 per cent GP with 1 per cent NSC and 1 per cent GP with 2 per cent NSC were 10.87 and 17.09 per cent respectively.

Raseena (2006) reported that mean serum total cholesterol was significantly lower in Azolla fed groups (147.79, 140.35 and 113.99 mg/dl, respectively for 1.5, 3 and 4.5 per cent inclusion) compared to control (167.92 mg/dl) in laying quails.

Uko and Kamalu (2006) conducted a study in which chicks were fed diets containing 75, 150 or 225 g autoclaved neem seed kernel (AFK/kg) or 75 g raw neem seed kernel (RFK/kg) offered as mash for 35 days. Result showed decrease in plasma cholesterol and triglyceride concentrations in chicks fed on diet containing RFK (142.4 and 86.2 mg/dl, respectively) compared to chicks placed on AFK (181.0 and 137.1; 183.7 and 134.4; 185.5 and 140.3 mg/dl, respectively) and control diet (194.3 and 130.5 mg/dl, respectively).

Dietary supplementation of turmeric at 0.4 and 0.6 per cent levels in diet of broiler chicken resulted in significant reductions in serum total cholesterol (166.02 and 157.44 mg/dl, respectively) as compared to control diet (175.89 mg/dl) fed group (Simi, 2007).

2.9.2 Serum protein

Supplementation of garlic powder, neem seed cake and their combinations did not influence the serum total protein values in six week old broiler chicken (Lonkar, 2006).

Raseena (2006) found that serum protein values were not influenced due to different levels of inclusion of Azolla (1.5, 3 and 4.5 per cent) in Japanese quail ration.

Uko and Kamalu (2006) conducted a study in which chicks were fed diets containing 75, 150 or 225 g autoclaved neem seed kernel (AFK/kg) or 75 g raw neem seed kernel (RFK/kg) offered as mash for 35 days. There were no significant differences among chicks fed on the reference or test diets for total protein, albumin and globulin.

The serum total protein values in the different dietary groups fed with turmeric at 0, 0.2, 0.4 and 0.6 per cent levels were 3.56, 3.68, 3.70 and 3.0 g/dl, respectively and were statistically comparable. (Simi, 2007)

2.9.3 Serum creatinine and uric acid

Buckingham *et al.* (1978) opined that a total of 5.5 per cent of the Azolla nitrogen was present as purine, resulting in a purine concentration of 492 mg/100g of Azolla, which is equally distributed between adenine and guanine.

Oloyede *et al.* (2004) reported that broiler birds fed on the raw melon seed meal and fermented melon seed meal diets had higher uric acid values (1.93 and 1.17 nmol/l, respectively) than those fed on the control diet containing soya bean meal and fermented cooked melon seed meal (0.46 and 0.93 nmol/l, respectively). They also observed that birds fed on raw melon seed meal and fermented melon seed meal based diets had slightly low values of creatinine (53.6 and 50.3 nmol/l, respectively) compared to soya bean meal and fermented cooked melon seed meal based diets (55.6 and 55 nmol/l, respectively).

Raseena (2006) observed that serum creatinine and uric acid values were not influenced due to inclusion of Azolla at levels of 1.5, 3 and 4.5 per cent in Japanese quail ration.

Uko and Kamalu (2006) conducted a study in which chicks were fed diets containing 75, 150 or 225 g autoclaved neem seed kernel (AFK/kg) or 75 g raw neem seed kernel (RFK/kg) offered as mash for 35 days. No significant differences were found among chicks fed on the reference or test diets for creatinine and uric acid values.

2.10 Effect on meat cholesterol

Lonkar (2006) studied the effect of garlic (*Allium sativum*) powder (GP) and neem (*Azadirachta indica*) seed cake (NSC) on cholesterol content in broiler chicken and reported that the supplementation of GP and NSC alone and their combinations significantly ($P \leq 0.05$) reduced the breast and thigh meat total cholesterol in broiler chicken.

Simi (2007) reported that supplementation of turmeric at 0.4 per cent resulted in comparatively lower level of thigh meat cholesterol (110.95 mg/dl) in broilers than that recorded in control group (113.08 mg/dl).

Shim *et al.* (2004) found that the amounts of total cholesterol in breast muscle were comparatively lower in broiler chicks fed diets containing *Codonopsis lanceolata* root at 0.25 per cent and 0.5 per cent (0.64 and 0.61 mg/g, respectively) than that in control fed chicks (0.65 mg/g).

2.11 Livability

Khatun *et al.* (1999) could not observe any mortality in laying hens when they were fed with diets containing 150 and 200 mg/kg Azolla meal based on a digestible protein and digestible amino acid basis.

Basak *et al.* (2002) observed cent per cent survivability in broiler chicks when they were fed with Azolla at different levels (5, 10 and 15 per cent).

Parthasarathy *et al.* (2002) reported that feeding of Azolla up to 20 per cent level did not have any adverse effect on the livability of broilers.

Alalade and Iyayi (2006) also reported that Azolla meal up to 15 per cent level in the diet of egg type chicks did not have any adverse effect on the livability of birds.

2.12 Economics

Singh and Subudhi (1978) suggested that due to high price of commercial feed, Azolla has a very good future in poultry feeding. They observed that a poultry farm with 100 birds will require about 9 kg fresh Azolla per day to replace 20 per cent of the commercial feed (1.1 kg feed/day).

Becerra *et al.* (1995b) concluded that fresh Azolla can replace whole soya beans up to a level of about 20 per cent of the total crude protein in diets based on sugar cane juice in fattening ducks. Cost of feed per kg gain was lowest and net profit per bird was highest for 20 per cent replacement of soya beans by fresh Azolla.

Jayaraman *et al.* (1995) studied the economics of integrated fish-duck-Azolla farming system and reported that feeding the fish and ducks with Azolla helped to reduce feed cost by 24 per cent besides enriching the egg yolk.

Men *et al.* (1996) reported that duckweed can be used as replacement for roasted soya beans in diet containing broken rice as the basal ingredient for fattening ducks and stated that for the farmers growing the duckweed, the feed costs per kg live weight gain were reduced substantially by feeding duckweed with the greatest benefit on the diet that contained no soya beans.

Parthasarathy *et al.* (2001a) studied the economics of utilizing Azolla in broiler ration at levels of 0, 5, 10, 15 and 20 per cent by replacing a protein mix containing wheat bran and groundnut cake (53:47) in first trial and wheat bran and fish meal (52:48) in second trial. The findings indicated that incorporation of Azolla as a feed ingredient at 5 per cent level in broiler rations could provide promising economic returns.

Basak *et al.* (2002) reported azolla meal is an unconventional feed ingredient at lower price and may be used up to 5 per cent level in broiler diet to reduce feed cost.

Khanum *et al.* (2005) conducted experiment to determine the intake and digestibility of fresh duckweed in growing ducks. They fed ducks with three diets (control diet, 50 per cent control diet + *ad libitum* fresh and harvested duckweed (DWH), 50 per cent control diet + ducks were allowed to graze on duckweed in lagoon (DWG)) and stated that replacement of 50 per cent of the control diet through feeding duckweed under confinement or through grazing fresh duck weed reduced the feed costs to farmers by about 50 per cent.

Khandaker *et al.* (2007) studied the effect of feeding duckweed that replaced conventional protein supplement mustard oil cake (MOC) in the diets for laying ducks. The diets were based on rice by products where soybean meal and mustard oil cake as protein source. The control diet A contained 15 per cent MOC and 5, 10 and 15 per cent MOC was replaced with dry duckweeds in the diets B, C and D respectively. They reported that by considering economic benefit, it may be recommended to replace Mustard oil cake by up to 15 per cent level in the diet of laying duck

MATERIALS AND METHODS

3. MATERIALS AND METHODS

An experiment was conducted at the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy to study the effect of dietary inclusion of dried Azolla on production performance, serum biochemical parameters and meat cholesterol content in broiler chicken. The study was conducted for a period of six weeks from 18 th October to 30 th November, 2007.

One hundred and sixty, day-old commercial broiler chicks (Vencob) were procured from Venketeswara Hatcheries (P) Ltd. Palakkad, Kerala. The chicks were wing banded and weighed individually. They were allotted randomly to four treatment groups with four replicates of ten birds each as detailed below.

Treatment	Number of replicates	Number of birds in each replicate	Inclusion of dried Azolla in diet (%)
T1	4	10	0.0
T2	4	10	1.5
T3	4	10	3.0
T4	4	10	4.5

The chicks were reared under deep litter system of management. Thorough cleaning and disinfection of the experimental shed was carried out prior to the commencement of the experiment. Litter materials were spread to a thickness of 6 cm in each pen. A floor space of 925 cm^2 / chick was allotted. Feeders, waterers and other equipment were cleaned, disinfected and sun dried before use.

The chicks were brooded till they attained four weeks of age. Thereafter light was provided during night hours to enhance the feed intake. Standard managemental procedures were followed during the experiment. Chicks were immunized against Ranikhet Disease and Infectious Bursal Disease. The birds were provided with feed and clean drinking water *ad libitum* throughout the experimental period.

Experimental ration

Broiler starter and finisher rations were formulated as per BIS (1992) specifications of nutrients for broiler chicken. Azolla was cultivated, multiplied and harvested in paddy field at Govt. Agricultural farm at Kachirayapalayam using a culture received from Tamil Nadu Agricultural University, Coimbatore. The Azolla were dried in sunlight after harvesting. Dried Azolla was incorporated in to the rations as per the levels indicated earlier. The rations were made iso-caloric and iso-nitrogenous. Broiler starter diet was fed up to four weeks of age. Broiler finisher diet was given during fifth and sixth weeks of age.

Proximate analysis of the feed ingredients and rations were done according to procedures described by AOAC (1990).

The ingredient composition of the four different starter and finisher rations is presented in Tables 1 and 2, respectively and the chemical composition of the four different starter and finisher rations is presented in Tables 3 and 4, respectively. The performance of birds was recorded for a period of six weeks. The following parameters were studied during the course of the experiment.

3.1 Meteorological data

The wet and dry bulb thermometer readings were taken at 8 AM and 2 PM daily. The maximum and minimum temperatures were recorded at forenoon on all days throughout the experimental period. From these data, weekly mean maximum and minimum temperatures and relative humidity were worked out.

3.2 Body weight and weight gain

The body weight of individual bird was recorded at weekly interval. From these data, the mean weekly body weight and weight gain were calculated for various treatment groups.

3.3 Feed intake

Feed intake of birds was recorded replicate wise at weekly interval. From these data the average intake per bird per week was calculated for various treatment groups.

3.4 Feed efficiency

Feed efficiency (kg of feed per kg weight gain) was calculated in each replicate based on the data on body weight gain and feed consumed.

3.5 Processing yields and losses

Two birds from each replicate were slaughtered at the end of experimental period to study the serum and meat biochemical characteristics and the processing yields and losses. The birds were fasted over night. Blood samples were collected without adding anticoagulant for serum collection. The jugular vein was severed and the birds were allowed to bleed for 2 min.

3.6 Serum biochemical parameters

3.6.1 Serum total cholesterol and serum total protein

Before slaughter, blood samples of two birds from each replicate were collected in clean dry labeled glass tubes. The tubes were kept in slanted position at room temperature to facilitate the separation of serum.

The serum total cholesterol was estimated colorimetrically by CHOD – PAP method utilizing the kit supplied by Agappe Diagnostics Pvt. Ltd., Ernakulam, Kerala – 683 562, India. The serum total protein was estimated colorimetrically by Direct biuret method utilizing the kit supplied by Agappe Diagnostics Pvt. Ltd., Ernakulam, Kerala – 683 562, India.

3.6.2 Serum creatinine and uric acid

The serum creatinine was estimated colorimetrically by modified Jaffe's method utilizing the kit supplied by Agappe Diagnostics Pvt. Ltd., Ernakulam, Kerala – 683 562, India. The serum uric acid was estimated colorimetrically by uricase – PAP method utilizing the kit supplied by Agappe Diagnostics Pvt. Ltd., Ernakulam, Kerala – 683 562, India.

3.7 Meat total cholesterol

At the time of slaughter, samples from breast and thigh muscles were collected. The breast and thigh muscles were preserved at – 18^0 C under deep freeze. The lipid was extracted by the method suggested by Folch *et al.* (1957) from breast and thigh muscles. The total cholesterol was estimated from extracted lipid by one step method of Wybenga *et al.* (1970) utilizing the kit supplied by Qualigens line chemicals, Dr.Anne Besant road, Worli, Mumbai – 400 025, India.

3.8 Livability

The mortality of birds from different treatment groups was recorded and the livability was worked out. Post mortem examination was conducted in each case to find out the cause of death.

3.9 Economics

Cost of feed for different dietary treatments was calculated based on the cost of ingredients. Cost of feed per kilogram live weight for different dietary treatments was calculated based on body weight attained and recurring expenditure at six weeks of age.

3.10 Statistical analysis

Data collected on various parameters were statistically analyzed as per the methods described by Snedecor and Cochran (1994).

Table 1. Per cent ingredient composition of experimental starter rations.

Sl.no	Ingredients	Starter ration			
		T1	T2	T3	T4
1	Yellow maize	54.50	54.50	54.50	55.75
2	Deoiled rice bran	5.50	4.50	3.50	1.00
3	Deoiled soyabean meal	24.00	23.50	23.00	22.75
4	Gingelly oil cake	4.00	4.00	4.00	4.00
5	Azolla	0.00	1.50	3.00	4.50
6	Dried fish	10.0	10.0	10.0	10.0
7	Mineral mixture[1]	1.75	1.75	1.75	1.75
8	Salt	0.25	0.25	0.25	0.25
	Total	100.0	100.0	100.0	100.0
Added per 100 kg feed					
1	Vitamin mixture[2],g	10	10	10	10
2	DL - methionine,g	120	120	120	120
3	L - lysine,g	140	140	140	140
4	Choline chloride[3],g	120	120	120	120
5	Coccidiostat[4],g	50	50	50	50
6	Toxin binder[5],g	100	100	100	100

[1]Mineral mixture composition : Calcium 32%, Phosphorus 6%, Magnesium1000pm, Cobalt 60 ppm, Zinc 2600ppm, Iron 0.1%, Iodine 100ppm, Copper 100ppm, Manganese 2700 ppm.

[2]Vitamin mixture, NICOMIX A, B_2, D_3, K. powder (Nicholas Primal India Ltd., Mumbai).

[3]Bio choline (Indian Herbs Research and supply Co. Ltd., U.P.) Containing Choline chloride 50%.

[4]Salinomix containing Salinomycin sodium 12.5% (Venkey's Pvt Ltd., Tamil Nadu).

[5] UTTP powder (Tetragon chemic Pvt, Ltd., Bangalore) containing treated Alumino silicates, Propionates, Formates, Acetates.

Table 2. Per cent ingredient composition of experimental finisher rations.

Sl.no	Ingredients	Finisher ration			
		T1	T2	T3	T4
1	Yellow maize	65.00	64.40	64.50	65.00
2	Deoiled rice bran	4.40	4.00	3.00	1.40
3	Deoiled soyabean meal	18.50	18.00	17.40	17.00
4	Azolla	0.00	1.50	3.00	4.50
5	Dried fish	10.0	10.0	10.0	10.0
6	Dicalcium phosphate	0.10	0.10	0.10	0.10
7	Mineral mixture[1]	1.75	1.75	1.75	1.75
8	Salt	0.25	0.25	0.25	0.25
	Total	100.0	100.0	100.0	100.0
Added per 100 kg feed					
1	Vitamin mixture[2],g	10	10	10	10
2	DL - methionine,g	120	120	120	120
3	L - lysine,g	140	140	140	140
4	Choline chloride[3],g	120	120	120	120
5	Coccidiostat[4],g	50	50	50	50
6	Toxin binder[5],g	100	100	100	100

[1]Mineral mixture composition : Calcium 32%, Phosphorus 6%, Magnesium1000pm, Cobalt 60 ppm, Zinc 2600ppm, Iron 0.1%, Iodine 100ppm, Copper 100ppm, Manganese 2700 ppm.

[2]Vitamin mixture, NICOMIX A, B_2, D_3, K. powder (Nicholas Primal India Ltd., Mumbai).

[3]Bio choline (Indian Herbs Research and supply Co. Ltd., U.P.) Containing Choline chloride 50%.

[4]Salinomix containing Salinomycin sodium 12.5% (Venkey's Pvt Ltd., Tamil Nadu).

[5] UTTP powder (Tetragon chemic Pvt, Ltd., Bangalore) containing treated Alumino silicates, Propionates, Formates, Acetates.

Table 3. Per cent chemical composition of experimental starter rations on DM basis.

Sl.no	Composition	Experiment ration			
		T1	T2	T3	T4
1	Moisture	10.98	11.02	11.09	10.83
2	Crude protein	22.60	22.40	23.16	22.80
3	Ether extract	3.05	3.10	3.14	3.22
4	Crude fibre	4.20	3.95	4.70	4.30
5	NFE	50.97	50.43	49.21	49.55
6	Total ash	8.2	9.1	8.7	9.3
7	AIA	2.3	2.3	2.2	2.1
Calculated values					
1	ME kcal / kg	2801	2796	2792	2804
2	Lysine	1.27	1.26	1.24	1.22
3	Methionine	0.50	0.49	0.49	0.50
4	Calcium	1.36	1.39	1.42	1.45
5	Total phosphorus	0.59	0.59	0.60	0.60

Table 4. Per cent chemical composition of experimental finisher rations on DM basis.

Sl.no	Composition	Experiment ration			
		T1	T2	T3	T4
1	Moisture	10.65	11.02	10.78	10.25
2	Crude protein	19.77	19.60	20.19	19.97
3	Ether extract	3.40	3.40	3.50	3.50
4	Crude fibre	3.8	3.5	3.4	4.1
5	NFE	53.88	53.08	53.43	53.28
6	Total ash	8.5	9.4	8.7	8.9
7	AIA	2.2	2.2	2.1	2.0
Calculated values					
1	ME kcal / kg	2909	2896	2893	2895
2	Lysine	1.06	1.05	1.03	1.01
3	Methionine	0.45	0.45	0.44	0.44
4	Calcium	1.21	1.24	1.27	1.30
5	Total phosphorus	0.59	0.60	0.60	0.60

RESULTS

4. RESULTS

The results of an experiment conducted to study the effect of dietary inclusion of dried Azolla on production performance of broiler chicken are presented in this chapter.

4.1 METEOROLOGICAL PARAMETERS

The data pertaining to microclimate viz., the mean maximum and minimum temperatures (^{0}C) and relative humidity (%) inside the experimental house during the study period, that is, from 18th October to 30th November, 2007 are presented in Table 5. During the course of experiment, the mean maximum temperature ranged from 27.00 to 31.86 ^{0}C with a mean value of 29.90 ^{0}C, while the mean minimum temperature ranged from 24.10 to 26.20 ^{0}C with a mean value of 24.75 ^{0}C. The RH (%) varied from 77.43 to 83.86 at 8 AM and 62.48 to 73.43 at 4 PM. The mean per cent relative humidity in the forenoon and afternoon during the course of the experiment were 79.07 and 65.78, respectively.

Table 5. Mean weekly meteorological data in the experimental house during the period from October to November, 2007.

Age in weeks	Temperature (^{0}C)		Relative Humidity (%)	
	Maximum	Minimum	Forenoon	Afternoon
1	27.00	24.10	83.86	73.43
2	27.85	24.92	78.28	63.81
3	30.90	26.20	78.00	64.00
4	31.39	24.48	78.57	64.05
5	30.37	24.14	77.43	62.48
6	31.86	24.65	78.29	66.90
Mean	29.90	24.75	79.07	65.78

4.2 NUTRITIVE VALUE OF AZOLLA

Proximate analysis of sun dried and ground Azolla used in this experiment is presented in Table 6. Chemical composition showed that it contained 89.70 per cent dry matter and 24.50 per cent crude protein (CP), 3.7 per cent ether extract (EE), 14.90 per cent crude fibre (CF), 39.90 per cent nitrogen free extract (NFE) and 17.00 per cent total ash on dry matter basis. The calcium and phosphorus content of Azolla were 2.14 and 0.44 per cent, respectively. The metabolisable energy value of Azolla was found to be 1807 kcal/kg. Fresh Azolla had moisture content of 93.55 per cent. Photographs of *Azolla pinnata* cultivation in paddy field and sun dried and ground Azolla is given in Fig.1a and Fig.1b respectively. Chemical composition of Azolla is graphically represented in Fig.2.

Table 6. Per cent chemical composition of sun dried and ground Azolla (on dry matter basis).

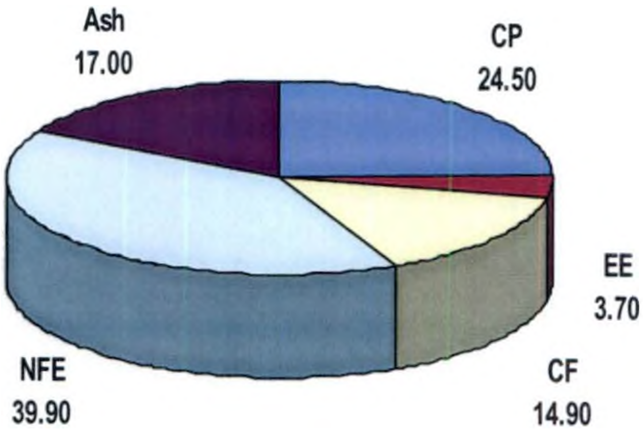
Sl.no	Components	Composition
	Dry matter	89.7
1	Crude protein	24.50
2	Ether extract	3.70
3	Crude fibre	14.90
4	Nitrogen free extract	39.90
5	Total ash	17.00
1	Calcium	2.14
2	Phosphorus	0.44
3	ME (kcal/ kg)*	1807

* Calculated value

Fig. 1a. ***Azolla pinnata*** **cultivation in paddy field**

Fig. 1b. Sun dried and ground Azolla

Fig.2. Per cent chemical composition of sun dried and ground Azolla (on dry matter basis)



4.3 BODY WEIGHT

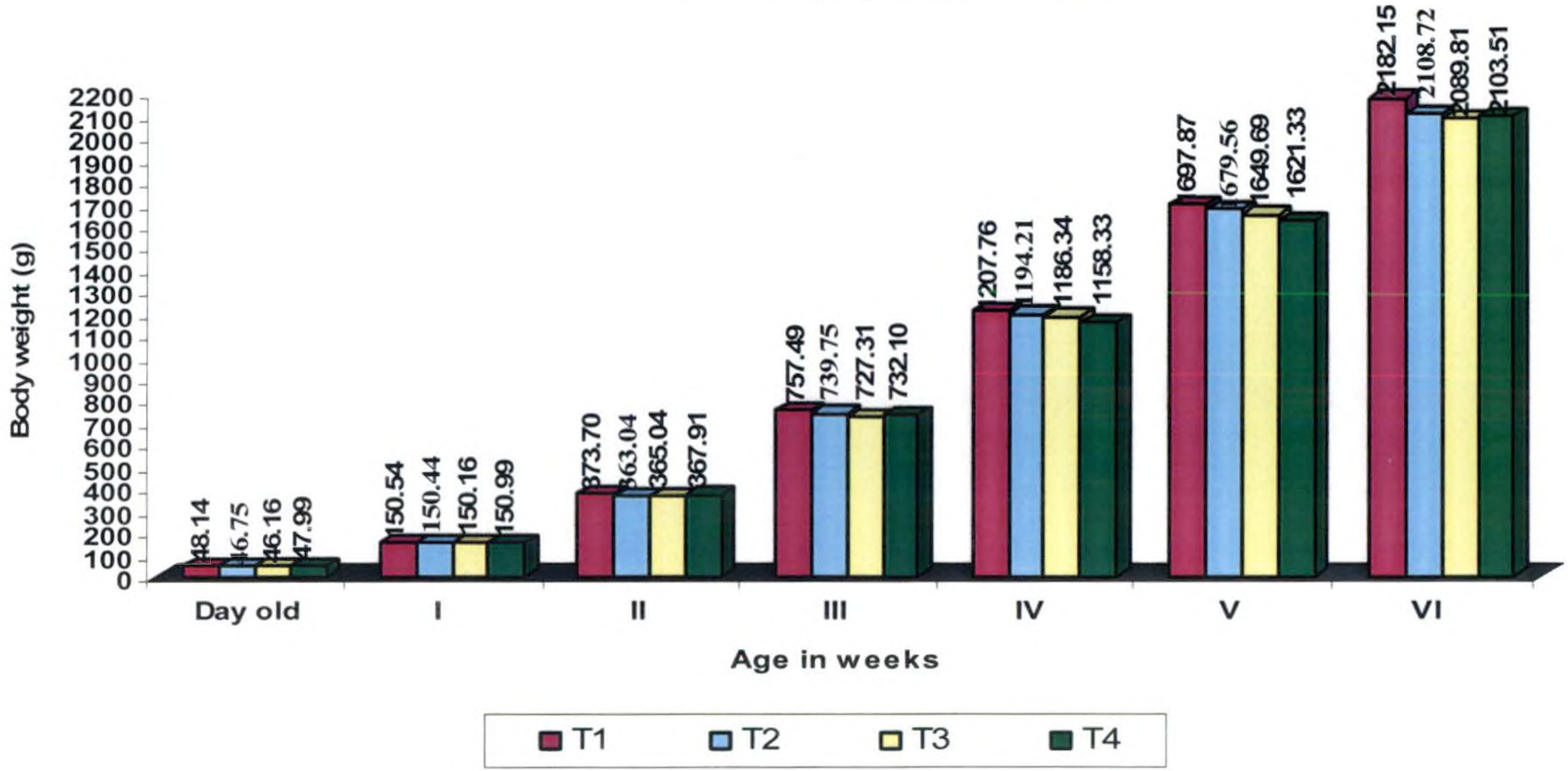
The data on mean body weight (g) of broiler chicken at weekly interval as influenced by different dietary treatments viz., control broiler ration (T1), broiler ration containing 1.5 per cent Azolla (T2), 3.0 per cent Azolla (T3) and 4.5 per cent Azolla are presented in Table 7 and graphically depicted in Figure 3. The day old body weight of chicks among different groups viz., T1, T2, T3 and T4 were 48.14, 46.75, 46.16 and 47.99 g, respectively with an overall mean of 47.30 g. The statistical analysis of the data on day old body weight of chicks did not reveal any significant difference among the treatment groups.

The mean body weight of chicks at the end of first week of age were 150.54, 150.44, 150.16 and 150.99 g, for the groups T1, T2, T3 and T4 respectively with an overall mean of 150.50 g. The mean body weight of chicks at the end of second week of age among the groups viz., T1, T2, T3 and T4 were 373.70, 363.04, 365.08 and 367.91 g, respectively with an overall mean of 367.40 g.

Table 7. Mean (± SE) weekly body weight (g) of broiler chicken as influenced by dietary inclusion of dried Azolla.

Age in weeks	Azolla levels (%) in Experimental Diets				Overall mean ± SE
	0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	
0	48.14 ± 1.00	46.75 ± 0.83	46.16 ± 1.06	47.99 ± 0.90	47.30 ± 0.29
I	150.54 ± 2.66	150.44 ± 2.51	150.16 ± 2.35	150.99 ± 2.27	150.50 ± 1.21
II	373.70 ± 6.25	363.04 ± 5.20	365.08 ± 5.83	367.91 ± 5.61	367.40 ± 2.86
III	757.49 ± 11.37	739.75 ± 9.98	727.31 ± 10.22	732.10 ± 8.26	739.20 ± 5.00
IV	1207.76 ± 18.12	1194.21 ± 17.38	1186.34 ± 20.32	1158.33 ± 14.34	1186.50 ± 8.68
V	1697.87 ± 27.75	1679.56 ± 27.93	1649.69 ± 32.58	1621.33 ± 25.29	1662.10 ± 13.88
VI	2182.15 ± 41.28	2108.72 ± 37.79	2089.81 ± 43.75	2103.51 ± 37.51	2122.60 ± 19.31

Fig. 3. Weekly mean body weight (g) of broiler chicken as influenced by inclusion of dried Azolla in experimental diets



The mean body weight of chicks at the end of third week of age among the treatments T1, T2, T3 and T4 were 757.49, 739.75, 727.31 and 732.10 g, respectively with an overall mean of 739.20 g. At the end of fourth week of age the mean body weight recorded among the groups viz., T1, T2, T3 and T4 were 1207.76, 1194.21, 1186.34 and 1158.33 g, respectively with an overall mean of 1186.50 g. The mean body weight of chicks at the end of fifth week of age were 1697.87, 1679.56, 1649.69 and 1621.33 g with an overall mean of 1662.10 g for the groups T1, T2, T3 and T4 respectively. Similarly, the mean body weight of chicks at the end of sixth week of age among the groups viz., T1, T2, T3 and T4 were 2182.15, 2108.72, 2089.81 and 2103.51 g, respectively with an overall mean of 2122.60 g.

The statistical analysis of the data on weekly mean body weight of chicks from one to six weeks of age did not reveal any significant difference between the treatment groups.

4.4 BODY WEIGHT GAIN

The data on mean body weight gain (g) of broiler chicken at weekly interval as influenced by inclusion of dried Azolla is given in Table 8 and graphically depicted in Figure 4. Mean body weight gain at the end of first week of age among the groups viz., T1, T2, T3 and T4 were 102.40, 103.69, 104.01 and 103.01 g, respectively with an overall mean of 103.3 g. The statistical analysis of the data did not reveal any significant difference among the dietary groups. At the end of second week of age, the mean body weight gain among the treatment groups viz., T1, T2, T3 and T4 were 223.16, 212.60, 214.92 and 216.92 g respectively with an overall mean of 216.90 g. Similar to the first week the statistical analysis of the data on body weight gain did not reveal any significant difference among the dietary groups during the second week also.

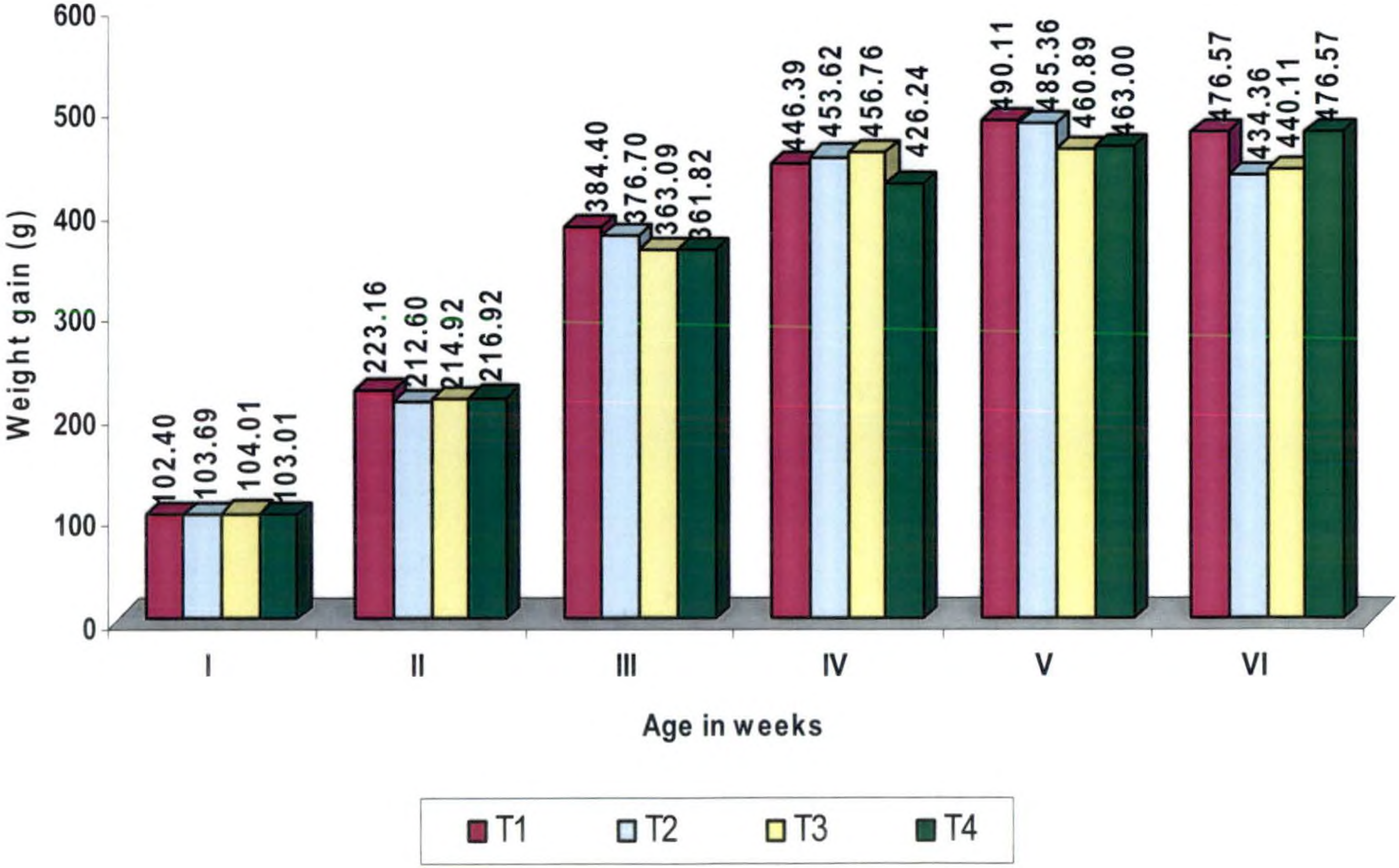
The mean body weight gain recorded at the end of third week of age among the groups T1, T2, T3 and T4 were 384.40, 376.70, 363.09 and 361.82 g, respectively with an overall mean of 371.50 g. Statistical analysis of this data revealed significant ($P \leq 0.05$) differences among the treatment groups. The birds maintained on 4.5 per cent (T4) and 3.0 per cent (T3) Azolla had statistically similar but significantly ($P \leq 0.05$) lower body weight gain than the birds maintained on control diet (T1). The birds fed 1.5 per cent Azolla (T2) had intermediate body weight which is statistically similar to all the other treatments.

Table 8. Mean (± SE) weekly weight gain (g) and cumulative weight gain (g) of broiler chicken as influenced by dietary inclusion of dried Azolla.

Age in weeks	Azolla levels (%) in Experimental Diets				Overall mean ± SE
	0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	
I	102.40 ± 2.55	103.69 ± 2.28	104.01 ± 2.10	103.01 ± 2.16	103.30 ± 1.13
II	223.16 ± 4.15	212.60 ± 3.90	214.92 ± 4.29	216.92 ± 3.91	216.90 ± 2.04
III	384.40^{b} ± 6.44	376.70^{ab} ± 7.64	363.09^{a} ± 7.27	361.82^{a} ± 6.00	371.50 ± 3.46
IV	446.39^{ab} ± 9.28	453.62^{ab} ± 9.94	456.76^{b} ± 11.83	426.24^{a} ± 8.92	445.70 ± 4.97
V	490:11 ± 13.28	485.36 ± 12.33	460.89 ± 15.34	463.00 ± 13.60	475.00 ± 6.65
VI	476.57 ± 17.21	434.36 ± 19.63	440.11 ± 16.72	476.57 ± 17.04	457.50 ± 8.58
Cumulative weight gain 0-4	1156.35 ± 30.11	1146.61 ± 20.97	1138.78 ± 30.48	1107.99 ± 25.92	1137.43 ± 13.51
Cumulative weight gain 0-6	2123.03 ± 70.17	2123.03 ± 52.54	2066.03 ± 73.98	2039.78 ± 60.17	2069.17 ± 32.29

Mean values bearing same superscript within a row did not differ significantly ($P \leq 0.05$)

Fig. 4. Weekly mean body weight gain (g) of broiler chicken as influenced by inclusion of dreid Azolla in experimental diets



The mean body weight gain recorded at the end of fourth week of age among different groups viz., T1, T2, T3 and T4 were 446.39, 453.62, 456.76 and 426.24 g respectively with an overall mean of 445.70 g. Statistical analysis of the data on fourth week body weight gain revealed significant ($P \leq 0.05$) differences among the treatment groups. The birds maintained on 3.0 per cent Azolla (T3) had significantly higher body weight gain than 4.5 per cent (T4) Azolla fed group. The weight gain of birds maintained on control diet and those fed with 1.5 per cent Azolla were intermediary and statistically similar to all the other treatment groups.

The mean weight gain of chicks at the end of fifth week of age among different groups viz., T1, T2, T3 and T4 were 490.11, 485.36, 460.89 and 463.00 g respectively with an overall mean of 475.00 g. The corresponding values at the end of sixth week of age among the groups T1, T2, T3 and T4 were 476.57, 434.36, 440.11 and 476.57 g respectively with an overall mean of 457.50 g.

The mean body weight gain at the end of fifth and sixth week of age for the birds in different dietary combinations did not differ significantly.

The mean cumulative body weight gain up to fourth week of age was 1156.35, 1146.61, 1138.78 and 1107.99 g with an overall mean of 1137.43 g. for treatments T1, T2, T3 and T4, respectively.

The mean cumulative body weight gain of birds up to sixth week of age for the above treatments was 2123.03, 2123.03, 2066.03 and 2039.78 g respectively with an overall mean of 2069.17 g. Statistical analysis of the data on cumulative weight gain up to fourth and sixth week of age did not reveal any difference among the dietary groups. Numerically higher cumulative weight gain at six weeks of age was recorded in the 1.5 per cent (T2) Azolla and control fed groups than the other groups.

4.5 WEEKLY FEED INTAKE

The data on mean feed intake at weekly interval as influenced by dietary inclusion of dried Azolla is given in Table 9 and graphically represented in Figure 5. Statistical analysis of the mean weekly feed intake data revealed that the dietary inclusion of different levels of dried Azolla had significant difference only at second and fifth week of age.

The mean weekly feed intake at the first week of age was 156.48, 156.05, 152.38 and 154.55 g for the treatments T1 to T4, respectively.

The mean weekly feed intake of chicks during the second week of age among different groups viz., T1, T2, T3 and T4 were 323.38, 338.45, 367.18 and 328.00 g respectively and statistical analysis of the data revealed significant ($P \leq 0.05$) differences among the treatment groups. The birds fed with 3.0 per cent Azolla had significantly higher feed intake than other treatment groups.

The mean weekly feed intake during the third and fourth of week of age for the treatments T1, T2, T3 and T4 were 581.23, 570.50, 563.05 and 580.50 and 742.13, 741.38, 738.78 and 730.03 g, respectively.

The data on mean weekly feed intake of birds recorded during fifth week of age among different groups viz., T1, T2, T3 and T4 age were 859.13, 885.55, 892.00 and 891.75 g, respectively. Statistical analysis of this data revealed significant ($P \leq 0.05$) differences among the treatment groups. The birds maintained on control group had significantly lower feed intake than Azolla fed groups.

The mean weekly feed intake during the sixth week of week of age for the groups T1, T2, T3 and T4 were 1014.33, 963.25, 945.68 and 1036.75 g, respectively.

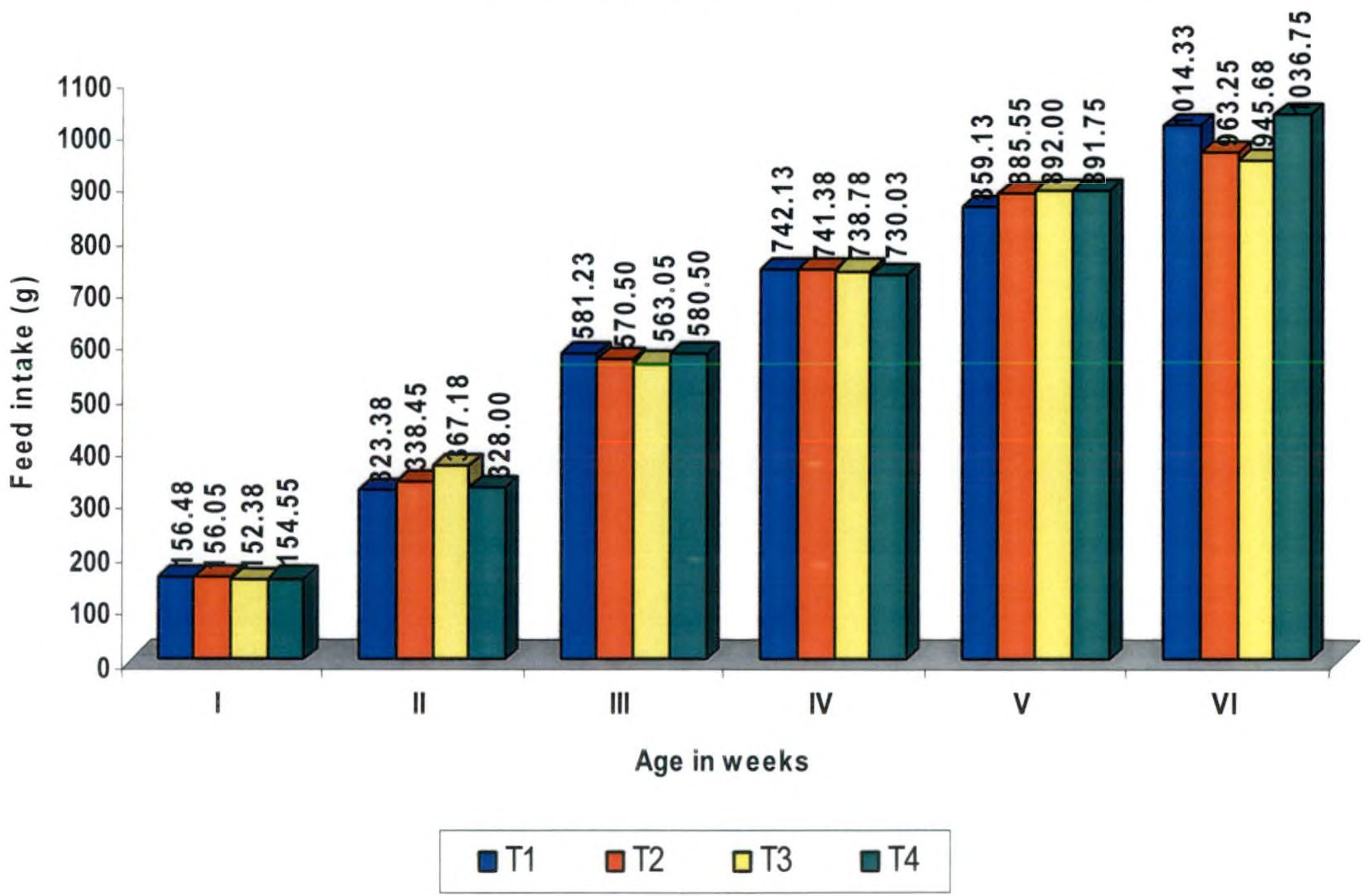
The over all mean weekly feed intake recorded during the period from one to six weeks of age irrespective of the treatments were 154.86, 339.25, 573.82, 738.08, 882.11 and 990.00 g, respectively.

Table 9. Mean (± SE) weekly feed intake (g) and cumulative feed intake (g) of broiler chicken as influenced by dietary inclusion of dried Azolla.

Age in weeks	Azolla levels (%) in Experimental Diets				Overall mean ± SE
	0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	
I	156.48 ± 0.64	156.05 ± 1.28	152.38 ± 2.60	154.55 ± 2.35	154.86 ± 0.94
II	323.38^a ± 4.76	338.45^a ± 7.66	367.18^b ± 2.15	328.00^a ± 4.64	339.25 ± 4.97
III	581.23 ± 10.41	570.50 ± 9.71	563.05 ± 12.96	580.50 ± 11.06	573.82 ± 5.33
IV	742.13 ± 6.44	741.38 ± 4.90	738.78 ± 8.24	730.03 ± 5.89	738.08 ± 3.15
V	859.13^a ± 7.31	885.55^b ± 5.98	892.00^b ± 4.69	891.75^b ± 4.21	882.11 ± 4.32
VI	1014.33 ± 40.73	963.25 ± 18.68	945.68 ± 33.92	1036.75 ± 9.98	990.00 ± 15.93
Cumulative F.I (0-4)	1803.20 ± 16.63	1806.38 ± 11.86	1821.38 ± 20.17	1793.09 ± 17.46	1806.01 ± 7.95
Cumulative F.I (0-6)	3676.65 ± 48.43	3655.18 ± 27.54	3659.05 ± 11.46	3721.59 ± 12.24	3678.12 ± 14.68

Mean values bearing the same superscript within a row did not differ significantly ($P \leq 0.05$)

Fig. 5. Weekly mean feed intake (g) of broiler chicken as influenced by inclusion of dried Azolla in experimental diets



The mean cumulative feed intake (Table 9 and Figure 6) of birds up to four weeks of age was 1803.20, 1806.38, 1821.38 and 1793.09 g for the treatments T1, T2, T3 and T4 respectively with an overall mean of 1806.01 g.

The mean cumulative feed intake of birds up to sixth week of age was 3676.65, 3655.18, 3659.05 and 3721.59 g for the treatments T1, T2, T3 and T4, respectively with an overall mean of 3678.12 g. Statistical analysis of the data on cumulative feed intake up to fourth and sixth week of age did not reveal any difference among the dietary groups.

4.6 DAILY FEED INTAKE

The data on mean daily feed intake as influenced by dietary inclusion of dried Azolla is given in Table 10. Statistical analysis of the mean daily feed intake data revealed that the dietary inclusion of dried Azolla at different levels had significant difference only at second and fifth week of age.

The mean daily feed intake of chicks at the first and second week of age among different groups viz., T1, T2, T3 and T4 were 22.35, 22.30, 21.75 and 22.08 and 46.18, 48.35, 52.45 and 46.88 g, respectively. The birds fed with 3.0 per cent Azolla had significantly higher ($P \leq 0.05$) feed intake during second week than other treatment groups.

The mean daily feed intake per bird during the third and fourth week of age were 83.03, 81.53, 80.45 and 82.95 g and 106.00, 105.93, 105.53 and 104.28 g for the groups T1, T2, T3 and T4, respectively. The mean daily feed intake of birds recorded during the fifth week of age among different groups viz., T1, T2, T3 and T4 age were 122.75, 126.48, 127.43 and 127.38 g respectively. The birds maintained on control group had significantly lower ($P \leq 0.05$) feed intake during fifth week of age than other Azolla fed groups.

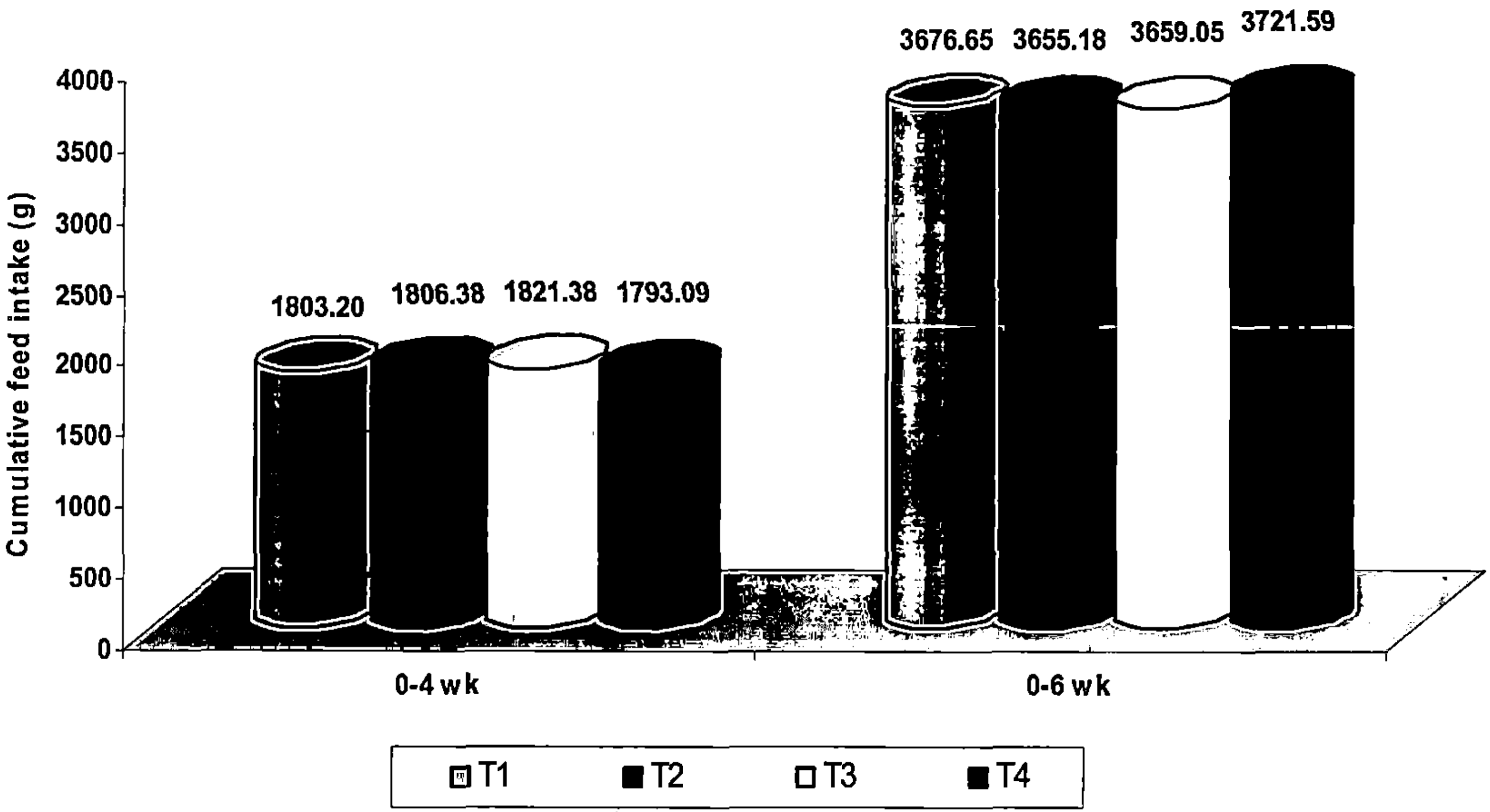
The mean daily feed intake of birds during the sixth week of age for the treatments T1, T2, T3 and T4 age were 144.93, 137.63, 135.10 and 148.10 g, respectively.

Table 10. Mean (± SE) daily feed intake (g) of broiler chicken as influenced by dietary inclusion of dried Azolla.

Age in weeks	Azolla levels (%) in Experimental Diets				Overall mean ± SE
	0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	
I	22.35 ± 0.10	22.30 ± 0.20	21.75 ± 0.38	22.08 ± 0.34	22.12 ± 0.31
II	46.18^a ± 0.68	48.35^a ± 1.08	52.45^b ± 0.31	46.88^a ± 0.66	48.46 ± 0.71
III	83.03 ± 1.48	81.53 ± 1.39	80.45 ± 1.86	82.95 ± 1.58	81.97 ± 0.76
IV	106.00 ± 0.92	105.93 ± 0.70	105.53 ± 1.19	104.28 ± 0.84	105.44 ± 0.45
V	122.75^a ± 1.03	126.48^b ± 0.85	127.43^b ± 0.68	127.38^b ± 0.59	126.02 ± 0.62
VI	144.93 ± 5.81	137.63 ± 2.66	135.10 ± 4.84	148.10 ± 1.41	141.43 ± 2.28

Mean values bearing same superscript within a row did not differ significantly ($P \leq 0.05$)

Fig. 6. Mean cumulative feed intake (g) of broiler chicken as influenced by inclusion of dried Azolla in experimental diets



4.7 FEED EFFICIENCY

The data on mean weekly feed efficiency of birds maintained on different dietary treatments is presented in Table 11. Statistical analysis of the mean feed efficiency values showed that the different dietary levels of Azolla had significant ($P \leq 0.05$) effect only during the second and fourth week of age and in the remaining weeks, the values were statistically comparable among treatments. The overall mean weekly feed efficiency during the period from one to six weeks of age, without considering the treatment effects, was 1.50, 1.57, 1.57, 1.64, 1.86 and 2.19 respectively.

The mean feed efficiency during the first week of age for the treatments T1, T2, T3 and T4 were 1.53, 1.51, 1.47 and 1.51, respectively. The mean feed efficiency of birds recorded at the second week of age for the treatments T1, T2, T3 and T4 were 1.45, 1.59, 1.71 and 1.52, respectively. Statistical analysis of second week feed efficiency data revealed significant ($P \leq 0.05$) differences among the treatment groups. The feed efficiency was best for control group (T1) and it was statistically similar to 4.5 per cent Azolla fed (T4) group. The birds fed with 3.0 per cent Azolla (T3) had significantly poor feed efficiency than control and 4.5 per cent Azolla fed (T4) group. However, it was statistically similar to 1.5 per cent Azolla fed (T2) group.

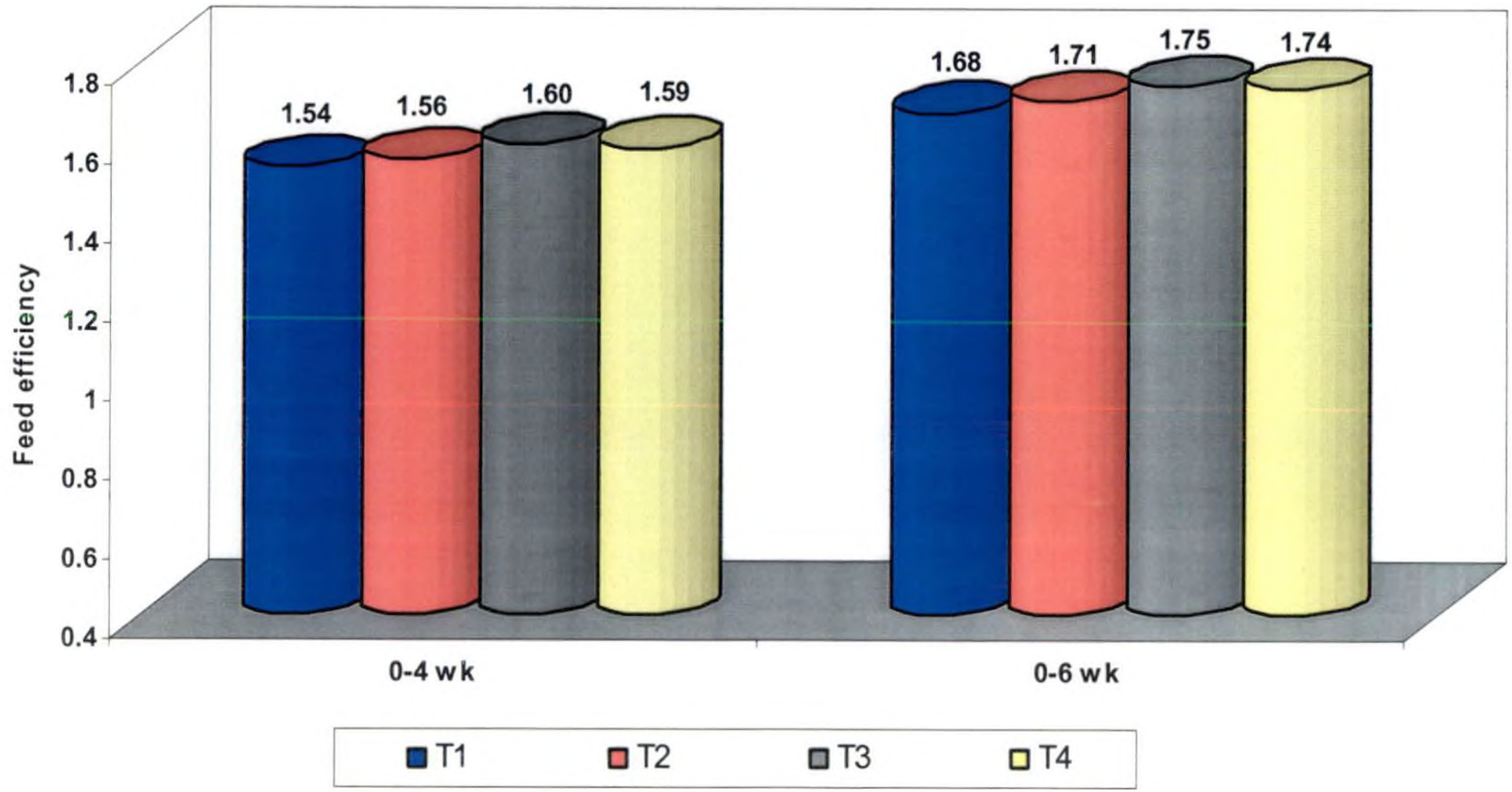
The mean feed efficiency of birds during the third week were 1.52, 1.52, 1.65 and 1.61 for the treatments T1 to T4, respectively. The mean feed efficiency of birds recorded at fourth week of age for the treatments T1, T2, T3 and T4 were 1.67, 1.63, 1.56 and 1.72, respectively. Statistical analysis of this data revealed significant ($P \leq 0.05$) differences among the treatment groups. The feed efficiency was superior for 3.0 per cent Azolla (T3) fed group and it was statistically similar to control group (T1) and 1.5 per cent Azolla fed (T2) group. The birds fed with 4.5 per cent Azolla (T4) had significantly inferior feed efficiency than those fed 3.0 per cent Azolla (T3). However, the groups T1, T2 and T4 were statistically comparable.

Table 11. Mean (± SE) weekly feed efficiency and cumulative feed efficiency of broiler chicken as influenced by dietary inclusion of dried Azolla.

Age in weeks	Azolla levels (%) in Experimental Diets				Overall mean ± SE
	0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	
I	1.53 ± 0.02	1.51 ± 0.03	1.47 ± 0.02	1.51 ± 0.04	1.50 ± 0.02
II	1.45^{a} ± 0.02	1.59^{bc} ± 0.05	1.71^{c} ± 0.03	1.52^{ab} ± 0.06	1.57 ± 0.03
III	1.52 ± 0.05	1.52 ± 0.04	1.65 ± 0.14	1.61 ± 0.07	1.57 ± 0.04
IV	1.67^{ab} ± 0.05	1.63^{ab} ± 0.03	1.56^{a} ± 0.03	1.72^{b} ± 0.02	1.64 ± 0.02
V	1.76 ± 0.06	1.83 ± 0.03	1.94 ± 0.08	1.93 ± 0.06	1.86 ± 0.03
VI	2.18 ± 0.09	2.21 ± 0.10	2.20 ± 0.09	2.20 ± 0.10	2.19 ± 0.04
Cumulative FCR (0-4)	1.54 ± 0.02	1.56 ± 0.01	1.60 ± 0.04	1.59 ± 0.04	1.57 ± 0.01
Cumulative FCR (0-6)	1.68 ± 0.03	1.71 ± 0.03	1.75 ± 0.04	1.74 ± 0.05	1.72 ± 0.02

Means bearing different superscript within a row differ significantly ($P \leq 0.05$)

Fig.7. Mean cumulative feed efficiency of broiler chicken as influenced by inclusion of dried Azolla in experimental diets



The mean weekly feed efficiency for the treatments T1, T2, T3 and T4 were 1.76, 1.83, 1.94 and 1.93 during the fifth week and 2.18, 2.21, 2.20 and 2.20 during the sixth week of age respectively.

The mean cumulative feed efficiency up to four and six weeks of age is set out in Table 11 and graphically depicted in Figure 7. The mean cumulative feed efficiency of birds up to four weeks of age calculated for the treatments T1, T2, T3 and T4 were 1.54, 1.56, 1.60 and 1.59, respectively with an overall mean of 1.57.

The mean cumulative feed efficiency of birds up to six weeks of age recorded for the treatments T1, T2, T3 and T4 were 1.68, 1.71, 1.75 and 1.74, respectively with an overall mean of 1.72. The statistical analysis of data on cumulative feed efficiency up to four and six weeks of age revealed no significant differences among the treatment groups.

4.8 PROCESSING YIELDS AND LOSSES

The mean per cent dressed, eviscerated and ready-to-cook yields and blood, feather and total losses recorded in broiler chickens slaughtered at six weeks of age as influenced by dietary inclusion dried Azolla are presented in Table 12 and graphically depicted figure 8a and 8b,respectively. The statistical analysis of the data on processing yields and losses revealed that dressed yield, eviscerated yield, ready to cook yield, feather loss and total loss did not differ significantly, while the blood loss and giblet yield differed significantly among the treatments ($P \leq 0.05$).

The mean per cent dressed, eviscerated and ready-to-cook yield for the treatments T1, T2, T3 and T4 were 91.85, 92.43, 92.14 and 92.10 and 69.88, 68.70, 68.95 and 71.24 and 74.01, 72.74, 72.76 and 75.60, respectively.

The mean per cent giblet yield recorded were 4.13, 4.08, 3.81 and 4.35 for treatments T1, T2, T3 and T4, respectively. The birds fed with 3.0 per cent Azolla (T3) recorded significantly lower giblet yield ($P \leq 0.05$) than that of 4.5 per cent Azolla fed (T4) group and both these groups were statistically comparable with the intermediatary values showed in control and T2 groups.

Table 12. Mean (± SE) per cent processing yields and losses in broiler chicken as influenced by dietary inclusion of dried Azolla.

Dietary Groups/ Azolla %	Yields				Losses		
	Dressed	Eviscerated	Giblet	Ready-to-cook	Blood	Feather	Total
T1 0.0	91.85 ± 0.61	69.88 ± 0.38	4.13^{ab} ± 0.12	74.01 ± 0.45	2.84^{a} ± 0.26	5.29 ± 0.47	25.99 ± 0.45
T2 1.5	92.43 ± 0.29	68.70 ± 1.69	4.08^{ab} ± 0.15	72.74 ± 1.67	3.23^{ab} ± 0.14	4.33 ± 0.24	27.26 ± 1.67
T3 3.0	92.14 ± 0.56	68.95 ± 1.60	3.81^{a} ± 0.19	72.76 ± 1.63	3.43^{ab} ± 0.28	4.46 ± 0.41	27.24 ± 1.63
T4 4.5	92.10 ± 0.50	71.24 ± 1.02	4.35^{b} ± 0.18	75.60 ± 0.94	3.84^{b} ± 0.34	4.11 ± 0.38	24.40 ± 0.94
Over all mean ± SE	92.10 ± 0.24	69.70 ± 0.64	4.10 ± 0.08	73.80 ± 0.64	3.30 ± 0.14	4.60 ± 0.20	26.20 ± 0.64

Means bearing the different superscript within a column differ significantly ($P \leq 0.05$)

Fig.8a. Mean per cent dressed and eviscerated yields of broiler chicken as influenced by inclusion of dried Azolla in experimental diets.

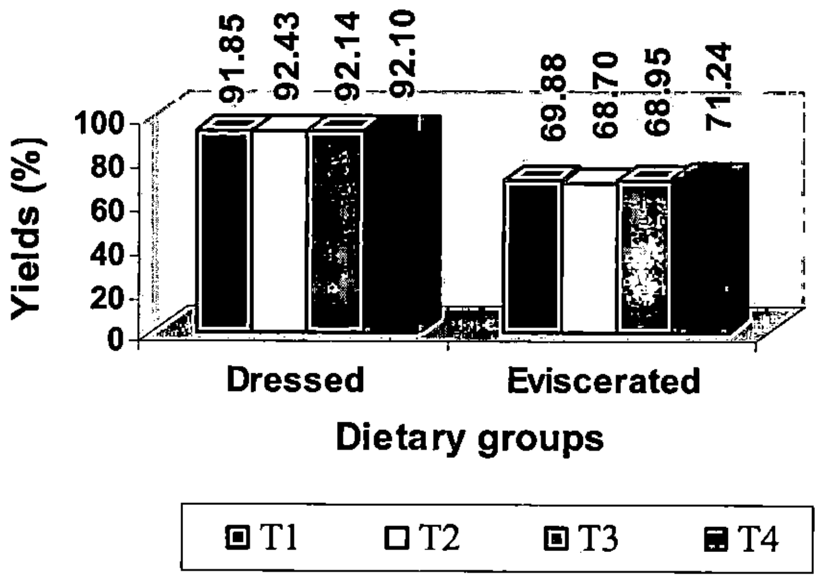
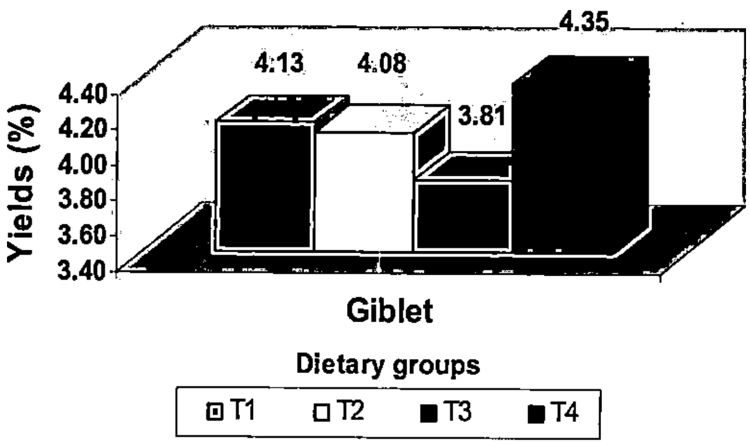


Fig.8b. Mean per cent giblet yield of broiler chicken as influenced by inclusion of dried Azolla in experimental diets.



The mean per cent feather and total losses for the treatments T1, T2, T3 and T4 were 5.29, 4.33, 4.46 and 4.11 and 25.99, 27.26, 27.24 and 24.40.

The mean per cent blood loss recorded for treatments T1, T2, T3 and T4 were 2.84, 3.23, 3.43 and 3.84, respectively. The birds fed with control diet had significantly lower blood loss than that of 4.5 per cent Azolla fed (T4) group (P≤0.05). On the other hand, per cent blood loss in 1.5 per cent Azolla (T2) and 3.0 per cent Azolla (T3) fed groups recorded intermediary values and were statistically comparable with both control and T4 groups.

4.9 SERUM PROFILES

4.9.1 Cholesterol

The mean serum total cholesterol (mg per dl) estimated at sixth week of age is set out in Table13 and graphically represented in Figure 9. The serum cholesterol levels at sixth week of age for the treatments T1, T2, T3 and T4 were 169.80, 169.71, 154.13 and 144.17 mg per dl respectively with an overall mean of 159.81 mg per dl. The statistical analysis of the mean serum total cholesterol values revealed significant differences among the treatments (P≤0.05).

The birds fed with 3.0 (T3) and 4.5 per cent Azolla (T4) fed groups had significantly (P≤0.05) lower serum cholesterol values (154.13 and 144.17 mg/dl, respectively) than that of control and 1.5 per cent Azolla (T2) fed groups (169.80 and 169.71 mg/dl, respectively).

4.9.2 Serum creatinine

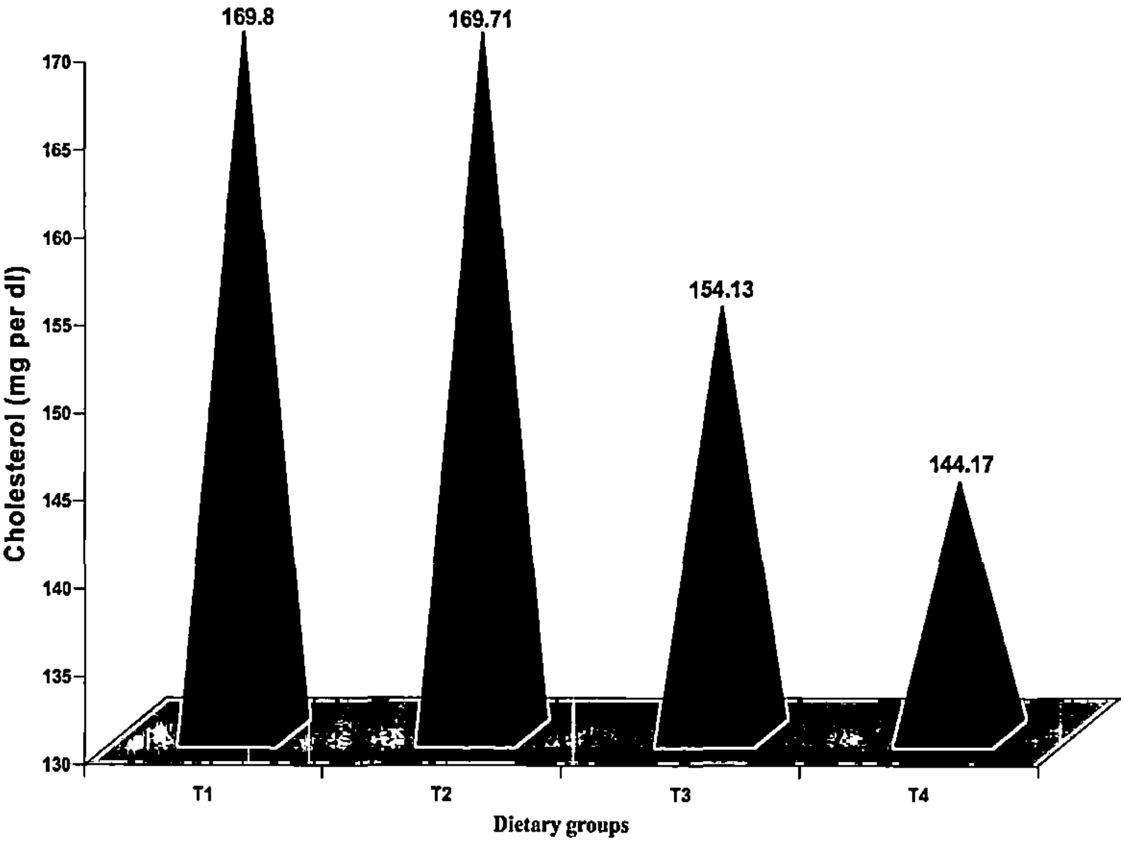
The mean serum total creatinine levels in broiler chickens estimated at the end of sixth week of age as influenced by dietary treatments are presented in Table 13. The mean serum creatinine levels (mg per dl) estimated for the treatments viz., T1, T2, T3 and T4 were 2.41, 2.61, 2.69 and 3.11, respectively with an overall mean of 2.71. Statistical analysis of the data on mean serum creatinine levels revealed significant differences among the treatments (P≤0.05). The birds fed on 4.5 per cent Azolla (T4) had significantly higher serum creatinine value than those of other treatment groups.

Table 13. Mean (± SE) serum total cholesterol (mg/dl), serum creatinine (mg/dl), serum total protein (g/dl) and serum uric acid (mg/dl) of broiler chicken as influenced by dietary inclusion of dried Azolla.

Sl. No.	Serum parameters	Azolla levels (%) in experimental diets				
		0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	Overall mean ± SE
1	Total cholesterol (mg/dl)	169.80^{b} ± 4.60	169.71^{b} ± 4.27	154.13^{a} ± 4.85	144.17^{a} ± 3.89	159.81 ± 2.94
2	Creatinine (mg/dl)	2.41^{a} ± 0.16	2.61^{a} ± 0.20	2.69^{a} ± 0.30	3.11^{b} ± 0.13	2.71 ± 0.08
3	Total protein (g/dl)	3.43 ± 0.34	3.09 ± 0.15	3.22 ± 0.11	3.39 ± 0.21	3.27 ± 0.08
4	Uric acid (mg/dl)	2.71 ± 0.24	2.71 ± 0.28	3.07 ± 0.22	3.17 ± 0.26	2.92 ± 0.09

Mean values bearing same superscript within a row did not differ significantly ($P \leq 0.05$)

Fig.9. Mean serum total cholesterol (mg/dl) value of broiler chicken as influenced by inclusion of dried Azolla in experimental diets.



4.9.3 Serum total protein

The mean serum total protein levels in broiler chickens estimated at the end of sixth week of age as influenced by different dietary treatments are presented in Table 13. The mean serum total protein levels (g per dl) determined for the treatments viz., T1, T2, T3 and T4 were 3.43, 3.09, 3.22 and 3.39 respectively with an overall mean of 3.27. Statistical analysis of the data on serum total protein did not reveal any significant difference among the treatments.

4.9.4 Serum uric acid

The mean serum uric acid levels in broiler chickens estimated at the end of sixth week of age as influenced by different dietary treatments are presented in Table 13. The mean serum uric acid levels (mg per dl) for the treatments viz., T1, T2, T3 and T4 were 2.71, 2.71, 3.07 and 3.17 respectively with an overall mean of 2.92. Statistical analysis of the data on mean serum uric acid levels did not reveal any significant difference among the treatments.

4.10 BREAST MEAT TOTAL CHOLESTEROL

The mean breast meat total cholesterol as influenced by dietary inclusion of dried Azolla at different levels estimated at the end of sixth week of age are presented in Table 14 and graphically depicted in Figure 10a. Statistical analysis of data revealed significant ($P \leq 0.05$) differences among the treatments. The highest breast meat cholesterol was observed in the control (T1) followed by 1.5 per cent (T2) and 3.0 per cent Azolla fed (T3) groups (74.45, 70.16 and 63.55 mg per dl, respectively), whereas the lowest value was noted in 4.5 per cent Azolla (T4) fed group (53.44 mg per dl). The maximum reduction in breast meat cholesterol was achieved in T4 followed by T3, T2 and T1 in descending order.

Table 14. Mean (± SE) breast and thigh meat total cholesterol of broiler chicken maintained on different dietary treatments.

Sl. No.	Parameters	Azolla levels (%) in experimental diets				
		0 T_1	1.5 T_2	3.0 T_3	4.5 T_4	Overall mean ± SE
1	Breast meat cholesterol (mg/dl)	74.45^{d} ± 1.29	70.16^{c} ± 0.83	63.55^{b} ± 0.88	53.44^{a} ± 1.07	65.40 ± 1.51
2	Thigh meat cholesterol (mg/dl)	122.75^{c} ± 0.86	122.78^{c} ± 0.97	116.84^{b} ± 0.85	105.54^{a} ± 1.19	116.96 ± 1.35

Means bearing the different superscript within the same column differ significantly ($P \leq 0.05$)

Fig.10a. Mean breast meat total cholesterol (mg/dl) of broiler chicken as influenced by inclusion of dried Azolla in experimental diets.

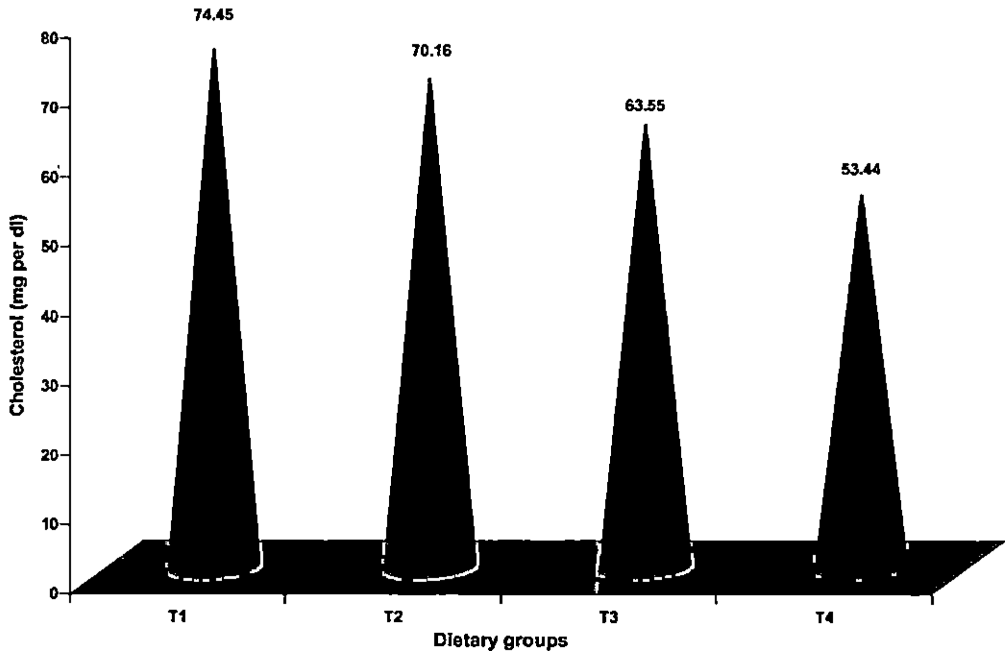
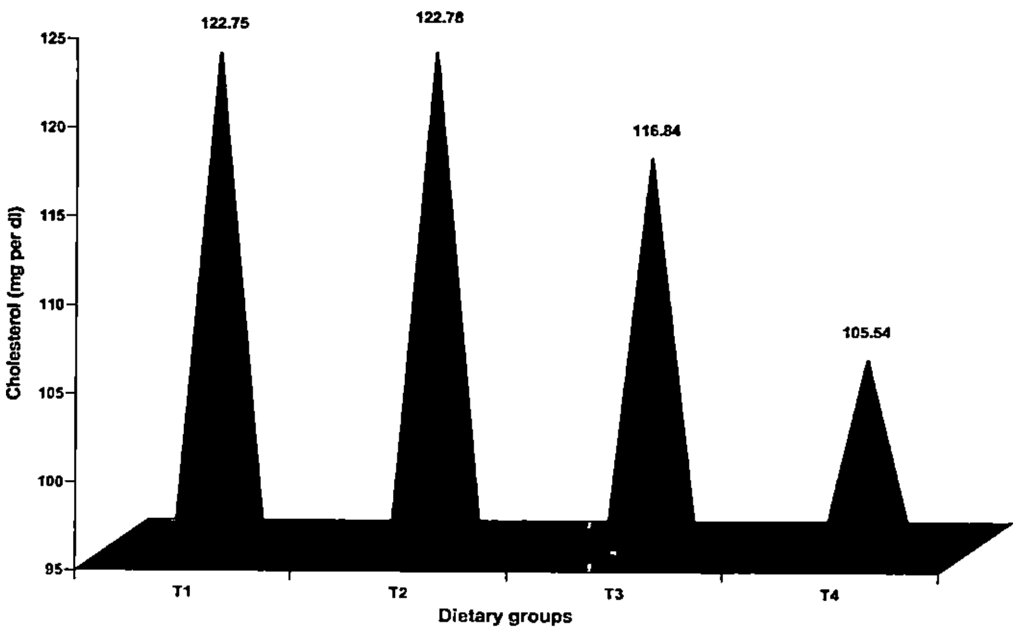


Fig.10b. Mean thigh meat total cholesterol (mg/dl) of broiler chicken as influenced by inclusion of dried Azolla in experimental diets.



4.11 THIGH MEAT TOTAL CHOLESTEROL

The mean thigh meat total cholesterol estimated at the end of sixth week of age is presented in Table 14 and graphically depicted in Figure 10b. The trend of result in this trait was comparatively similar to that of breast meat cholesterol. Statistical analysis of data revealed significant ($P \leq 0.05$) differences among the treatments. The highest thigh meat cholesterol was observed in the control (T1) and 1.5 per cent Azolla fed (T2) groups (122.75 and 122.78 mg per dl, respectively), whereas the lowest value was noted in the 4.5 per cent Azolla (T4) fed group (105.54 mg per dl). The 3.0 per cent Azolla fed group (T3) had intermediary thigh meat cholesterol level of 116.84 mg per dl. The maximum reduction of breast meat cholesterol among Azolla fed groups was achieved in T4 followed by T3 and T2 in descending order.

4.12 LIVABILITY

The mean week wise livability per cent as influenced by dietary inclusion of dried Azolla is presented in Table 15. The livability of birds in all the four treatments were comparatively similar up to two weeks of age. However, at the end of the experiment birds in 3.0 per cent and 4.5 per cent Azolla fed groups had slightly lower livability percentage than control group and 1.5 per cent Azolla fed group.

4.13 ECONOMICS

The costs of starter ration used in this study were 11.64, 11.52, 11.40 and 11.35 and that of finisher ration were 11.30, 11.25, 10.93 and 10.94 rupees per kg feed for the treatments T1, T2, T3 and T4 respectively. In order to calculate the cost of starter and finisher rations used in this experiment, the tender rates prevailed in the College of Veterinary and Animal Sciences for feed ingredients at the time of experiment were taken.

The economics of rearing broiler chicken by dietary inclusion of different levels of dried Azolla was worked out and given in Table 16. The average cost of production and total return from a bird at sixth week of age was calculated to assess the cost benefit.

The cost of production included cost of starter and finisher rations, chick cost and miscellaneous cost. The miscellaneous expenditure includes vaccination, medication and litter cost. The birds are sold at the rate of Rs. 45 per kg live weight. Cost of poultry manure also accounted for the total return. The net profit per kg body weight at sixth week of age was Rs. 17.57, 16.87, 16.99 and 16.88 for the treatments T1, T2, T3 and T4 respectively. The net profit per kg body weight was more in the birds fed with the control diet. Among the Azolla supplemented groups the net profit per kg body weight was more (Rs.16.99) in birds fed with 3.0 per cent Azolla (T3) followed by treatments T4 (Rs. 16.88) and T2 (Rs. 16.87).

Table 15. Mean weekly livability per cent as influenced by dietary inclusion of dried Azolla.

Age in weeks	Livability (%)				Overall mean
	T_1 Azolla (0 %)	T_2 Azolla (1.5 %)	T_3 Azolla (3.0 %)	T_4 Azolla (4.5 %)	
1	100.00	100.00	100.00	100.00	100.00
2	100.00	100.00	100.00	100.00	100.00
3	97.50	100.00	97.50	97.50	98.13
4	97.43	97.50	97.43	100.00	98.09
5	100.00	100.00	94.74	100.00	98.69
6	100.00	97.43	100.00	94.87	98.08
Cumulative livability (0-6)	95.00	95.00	90.00	92.50	93.13

Table 16. Cost benefit analysis for the different treatment groups at the end of sixth week of the experiment.

Particulars	Dietary treatments			
	T1	**T2**	**T3**	**T4**
Live body weight, g (6wk)	2182.15	2108.72	2089.81	2103.51
Starter feed intake/bird, g (0–4 wk)	1803.22	1806.38	1821.38	1793.09
Finisher feed intake/bird, g (5–6 wk)	1873.46	1848.80	1837.68	1928.75
Starter feed cost/kg feed, Rs	11.64	11.52	11.40	11.35
Finisher feed cost/kg feed, Rs	11.30	11.25	10.93	10.94
Total starter feed cost, Rs.	20.99	20.81	20.76	20.35
Total finisher feed cost, Rs.	21.17	20.80	20.08	21.10
Total feed cost, Rs	42.16	41.61	40.84	41.45
Chick cost, Rs.	14.50	14.50	14.50	14.50
Miscellaneous cost, Rs.	4.0	4.0	4.0	4.0
Total cost, Rs/bird	60.66	60.11	59.34	59.95
Return from bird sale, Rs @ Rs. 45 per kg	98.20	94.89	94.04	94.66
Return from manure sale, Rs	0.80	0.80	0.80	0.80
Net profit per bird, Rs	38.34	35.58	35.50	35.51
Net profit/kg body weight, Rs.	17.57	16.87	16.99	16.88

DISCUSSION

5. DISCUSSION

The results obtained from the study to find out the effect of inclusion of dried Azolla on the production performance of broiler chicken are discussed in this chapter.

5.1 METEOROLOGICAL PARAMETERS

The data pertaining to microclimate inside the experimental house (Table 5) showed that the mean maximum temperature was lowest (27.00^{0}C) during the first week and the highest (31.86^{0}C) during the last week of the biological trial. The difference in mean maximum temperature between the lowest and highest was only 4.86^{0}C. Similarly, the mean minimum temperature was lowest (24.10^{0}C) during the first week and highest (26.20^{0}C) during the third week of the trial. Mean maximum temperature showed a gradual raise from first to fourth week, then declined in the fifth week and further increased in the sixth week of the experiment. Whereas, the mean minimum temperature raised from first week to third week and then declined during the fourth and fifth week and again raised gradually and reached a temperature of 24.65^{0}C during the end of the experiment. Likewise, the mean per cent relative humidity in the forenoon and afternoon were 79.07 and 65.78 respectively. The results were in accordance with the findings of Somanathan (1980) who indicated that climatograph of this locality fell within the hot and humid climate.

5.2 NUTRITIVE VALUE OF AZOLLA

Chemical analysis of sun dried and ground Azolla presented in Table 6 indicated that it contained 24.50 per cent CP, 3.70 per cent EE, 14.90 per cent CF, 39.90 per cent NFE, 17.00 per cent ash, 2.14 per cent calcium and 0.44 per cent phosphorus on dry matter basis. The metabolisable energy content of Azolla was calculated as 1807 kcal/kg. Fresh Azolla contained 93.55 per cent moisture. The high volume of water present in Azolla limits the quantity able to be consumed by poultry and therefore, significantly reduces the amount of effective nutrient intake. Drying reduced the volume of Azolla considerably and concentrated the nutrients (Haustein *et al.*, 1990). Therefore, the present experiment was conducted with dried and ground Azolla.

The crude protein content of Azolla estimated in the present study (24.50 per cent) is in close agreement with the results of Buckingham *et al.* (1978), Subudhi and Singh (1978), Parthasarathy *et al.* (2001a), Basak *et al.* (2002), Alalade and Iyayi (2006) and Raseena (2006), who reported the crude protein content of Azolla meal in the range of 21.4 to 25.78 per cent. On the other hand, Sanginga and Van Hove (1989), Becerra *et al.* (1995b) and Khatun *et al.* (1999) observed higher CP content in Azolla meal (26.7 to 28.0 %). Contrary to this, Ali and Leeson (1995) and Ardakani *et al.* (1996) obtained only a lower CP content of 16.5 to 17.67 per cent in Azolla meal.

The 14.90 per cent CF obtained in the present study is in line with the findings of Querubin *et al.* (1986) and parthasarathy *et al.* (2001b) who reported the crude fibre content of Azolla in the range of 13.19 to 16.54 per cent. However, Ardakani *et al.* (1996) reported a higher crude fibre content of 21.5 per cent in Azolla. Contrary to this, Becerra *et al.* (1995b), Ali and Leeson (1995), Khatun *et al.* (1999), Alalade and Iyayi (2006) and Raseena (2006) could obtain only lower crude fibre content of 11.0 to 12.70 per cent.

The ether extract content of Azolla in the present study (3.70 per cent) is almost similar to the earlier observations of Subudhi and Singh (1978), Basak *et al.* (2002) and Raseena (2006), who reported an EE content of 3.5 to 3.7 per cent. But Buchingham *et al.* (1978) and Becerra *et al.* (1995b) reported higher values (5.05 and 4.6 %, respectively) of EE. However, Ali and Leeson (1995), Ardakani *et al.* (1996) and Alalade and Iyayi (2006) got lower EE values (1.60 to 2.70 per cent) than that obtained in the present study.

The ash content of Azolla obtained in this experiment is 17.0 per cent. Ali and Leeson (1995) reported a very high value of 36.12 per cent ash in Azolla. However, Buckingham *et al.* (1978), Parthasarathy *et al.* (2001b), Basak *et al.* (2002), Alalade and Iyayi (2006) and Raseena (2006) recorded values almost similar to the present study.

The NFE content of 39.90 per cent recorded in this study is consistent with the findings of Parthasarathy *et al.* (2001b), who reported 38.85 to 44.06 per cent NFE in Azolla. But Ali and Leeson (1995) observed a high value of 47.8 per cent NFE in Azolla.

The levels of calcium and phosphorus in the present study were found to be 2.14 and 0.44 per cent, respectively. On screening the literature, it was observed that the calcium and phosphorus contents of Azolla varied widely among different studies. The calcium level of Azolla obtained in this study was in close conformity with the report of Parthasarathy *et al.* (2001b), who indicated that *Azolla microphylla* contained 2.11 per cent calcium. Similarly, Ali and Leeson (1995) found 0.31 per cent phosphorus in Azolla, which was nearer to the result of the present study. Raseena (2006) also got similar values of calcium and phosphorus (2.29 and 0.39 per cent, respectively) in Azolla meal.

The variations in the nutrient composition of Azolla meal in different studies could be attributed to differences in the response of Azolla strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their growth morphology and composition. Moreover, species difference of Azolla could alter their nutrient composition. Furthermore, contamination with epiphytic algae could also be important to such a degree as to affect the results of chemical composition (Sanginga and Van Hove, 1989).

The results of present study confirm previous observations that Azolla was rich in proteins and minerals and that it could be used as a feed ingredient in poultry diets (Becerra *et al.* 1990).

5.3 BODY WEIGHT

The data on mean body weight of broiler chicken at weekly interval as influenced by different dietary treatments viz., control broiler ration (T1) and broiler rations containing 1.5 (T2), 3.0 (T3) and 4.5 per cent (T4) Azolla are presented in Table 7. The statistical analysis of the data on mean weekly body weight of chicks from one to six weeks of age did not reveal any significant difference between the treatment groups.

The results of this study indicated that inclusion of Azolla at levels of 1.5, 3.0 and 4.5 per cent in rations did not have any influence on weekly body weight in broiler chicken throughout the experimental period. This result is in agreement with the findings of Parthasarathy *et al.* (2002), who reported that broiler chicken fed with 5 per cent Azolla diets replacing 2.6 per cent wheat bran and 2.4 per cent fish meal gained more or less similar body weight to that of control at 8 weeks of age. On contrary, Basak *et al.* (2002) observed significant ($P \leq 0.01$) improvement in live weight of broiler chicks than control diet fed birds when they were fed with 5 per cent Azolla meal by replacing seasame meal. They also observed that use of higher levels of Azolla meal (10 and 15 per cent) resulted in poor growth rate than control diet fed groups.

Raseena (2006) also reported that the mean body weight of layer quails at twenty six weeks of age fed with Azolla at 0, 1.5, 3.0 and 4.5 per cent levels were 222.27, 223.31, 220.52 and 223.30 g, respectively. The statistical interpretation of the results showed no significant difference in body weight among different dietary treatment groups.

The inclusion of Azolla at higher levels limited its efficient utilization by monogastric animals as it contained ADF and lignin as indicated by Buckingham *et al.* (1978).

The results of this study revealed that dried Azolla can be added up to 4.5 per cent level in the broiler ration without any adverse effect on body weight of broiler chicken.

5.4 BODY WEIGHT GAIN

Statistical analysis of the mean weekly body weight gain data given in Table 8 revealed that the dietary inclusion of dried Azolla in different levels had significant effect only at third and fourth week of age.

At third week of age the birds maintained on 4.5 per cent (T4) and 3.0 per cent (T3) Azolla had significantly ($P \leq 0.05$) lower body weight gain than the birds maintained on control diet. The body weight gain in 1.5 per cent Azolla fed group was intermediary and was not significantly different from other groups. The reduction in body weight gain due to supplementation of Azolla at 1.5, 3.0 and 4.5 per cent levels was 7.7, 21.31 and 22.58 g, respectively. At fourth week of age the birds fed with 3.0 per cent Azolla significantly ($P \leq 0.05$) gained more weight (456.76 g), whereas, the 4.5 per cent Azolla fed groups gained less ($P \leq 0.05$) weight (426.24 g). The body weight gain of control birds and group fed 1.5 per cent Azolla were intermediary (446.39 and 453.62 g, respectively) and were statistically comparable with the other two groups.

The mean cumulative body weight gain of birds up to fourth and sixth week of age was 1156.35, 1146.61, 1138.78 and 1107.99 and 2123.03, 2123.03, 2066.03 and 2039.78 g for treatments T1, T2, T3 and T4 respectively. Statistical analysis of the data on cumulative body weight gain up to fourth and sixth week of age did not reveal any difference between the dietary groups. However, there was numerical reduction in body weight gain due to Azolla feeding at higher levels. The reduction in gain up to four weeks of age was to the tune of 9.74, 17.57 and 48.36 g for the treatments T2, T3 and T4 respectively. The reduction in weight gain up to six weeks of age was 0, 57.0 and 83.25 g respectively for 1.5, 3.0 and 4.5 per cent Azolla inclusion. This showed that a slight reduction in weight gain due to 1.5 per cent Azolla feeding up to four weeks of age was fully compensated when the birds attained six weeks of age. However, similar trend could not be seen with other Azolla levels. The results of the present study was in close agreement with those of Parthasarathy *et al.* (2001a), wherein broiler birds received 5 per cent Azolla from 0 to 5 weeks of age had similar weight gain as that of control, while the birds on 10, 15 and 20 per cent Azolla diets had significantly poor weight gain. Parthasarathy *et al.* (2002) also reported that 10 and 20 per cent Azolla inclusion in broiler rations resulted a significant reduction in live weight gain. However, Alalade and Iyayi (2006) could not observe any significant difference in weight gain of chicks fed with Azolla meal at the levels of 0, 5, 10 and 15 per cent in diet. Raseena (2006) also reported that mean body weight gain in layer quails was not differed statistically due to Azolla supplementation at 1.5, 3.0 and 4.5 per cent levels.

The results of the present study revealed that feeding of dried Azolla up to 4.5 per cent level did not have any adverse effect on body weight gain of broiler chicken.

5.5 FEED CONSUMPTION

The data on mean feed intake at weekly intervals as influenced by dietary inclusion of dried Azolla given in Table 9 indicated that this trait was not statistically different among treatments except in the second and fifth week of age.

The mean weekly feed intake of chicks during the second week of age among the groups viz., T1, T2, T3 and T4 were 323.38, 338.45, 367.18 and 328.00 g respectively. The birds fed with 3.0 per cent Azolla had significantly ($P \leq 0.05$) higher feed intake than those of other treatment groups.

Similarly, the analysis of the data on mean weekly feed intake of birds during the fifth week of age revealed significant ($P \leq 0.05$) differences among the treatment groups. The birds maintained on control feed had significantly lower feed intake (859.13 g) than 1.5, 3.0 and 4.5 per cent Azolla fed groups (885.55, 892.00 and 891.75 g respectively).

The mean cumulative feed intake of birds up to sixth week of age was 3676.65, 3655.18, 3659.05 and 3721.59 g for treatments T1, T2, T3 and T4 respectively. Statistical analysis of the data did not reveal any difference between the dietary groups.

However, numerical differences existed among treatments in 0 to 4 weeks and 0 to 6 weeks cumulative feed intake. An assessment of first four weeks cumulative feed intake due to Azolla inclusion showed that birds fed with 4.5 per cent Azolla consumed 10.11 g less feed than that of control birds. On the other hand, birds in the 1.5 and 3.0 per cent Azolla groups consumed 3.18 and 18.18 g, respectively more feed than the control group.

While considering the 0 to 6 weeks cumulative feed intake it was found that birds in the 4.5 per cent Azolla fed group compensated their lesser feed intake during 0 to 4 weeks period by consuming 44.94 g more feed than the control. It was also noted that birds offered 1.5 and 3.0 per cent Azolla fed groups consumed 21.47 and 17.60 g, respectively less feed than the control. No definite trend could be noted in cumulative feed intake due to Azolla feeding. Although, sixth week body weight was numerically more in control birds, it was not reflected in their cumulative feed intake. In general, the difference in cumulative feed intake between control and Azolla supplemented groups, is less than 45 g only.

Similar to mean weekly feed consumption, the mean daily feed intake as influenced by dietary inclusion of various levels of dried Azolla had significant differences only at second and fifth week of age (Table 10).

The birds fed with 3.0 per cent Azolla had significantly higher ($P \leq 0.05$) daily feed intake than other treatment groups during second week. Whereas, in the fifth week, all the Azolla fed groups consumed significantly ($P \leq 0.05$) more feed than control birds. However, the difference in feed intake between control birds and Azolla fed groups was less than 4.69 g only.

Absence of significant influence on mean weekly cumulative feed intake due to Azolla supplementation in broiler chicken as observed in the present study is in agreement with the findings of Ali and Leeson (1995), Sarria and Preston (1995) and Basak *et al.* (2002). They could not observe any significant difference in cumulative feed intake between Azolla fed and control diet fed broiler chicken. Similarly, Raseena (2006) also concluded that layer quail diets supplemented with 1.5, 3.0 and 4.5 per cent Azolla did not have significant influence on cumulative feed intake. On the other hand, Parthasarathy *et al.* (2001a) reported that mean cumulative feed intake was decreased when Azolla was increased from 0 to 15 per cent, but slight increase in feed intake was observed at 20 per cent level. Alalade and Iyayi (2006) also observed that 10 and 15 per cent Azolla meal in the diet of chicks significantly reduced the average weekly feed intake. But average weekly feed intake was similar, up to 5 per cent Azolla meal inclusion in diets, to that of control diet. Querubin *et al.* (1986) also studied the feeding value of Azolla meal in broiler chicks at levels of 0, 5, 10 and 15 per cent on dry matter basis. Average feed consumption of the birds increased significantly at 5 per cent level and also consistently increased with higher levels of Azolla meal in the diet.

Differences in feed intake with addition of varying levels of Azolla in poultry rations observed by researchers are not consistent. Variation in feed intake with changes in dietary ingredients as supported by Isshiki and Nakahiro (1989) could be the possibility for the numerical increase in daily feed intake noted in this study with Azolla fed groups. They stated that passage rate and feed intake changed based on type of feed ingredients used in formulating the diets. But, Bested and Morento (1985) stated that Azolla affected the palatability of the feed and reduced feed consumption.

Hence it can be conclusively stated from the results of this study that Azolla meal in broiler chicken ration up to 4.5 per cent level did not have any influence on feed intake.

5.6 FEED EFFICIENCY

Statistical analysis of the mean weekly feed efficiency values showed that the different dietary levels of Azolla had significant ($P \leq 0.05$) effects only during the second and fourth week of age and in the remaining weeks, the values were statistically comparable among treatments (Table 11). In the second week the feed efficiency was best for control (T1) group (1.45) and it was statistically similar to 4.5 per cent Azolla fed (T4) group (1.52). The birds fed with 3.0 per cent Azolla (T3) had significantly inferior feed efficiency (1.71) than control and 4.5 per cent Azolla fed (T4) groups. Although the feed efficiency of 1.5 per cent Azolla (T2) fed group (1.59) was poorer than control, the same was statistically similar to other Azolla supplemented groups during second week of age.

The mean feed efficiency of birds calculated for the fourth week of age for the treatments T1, T2, T3 and T4 were 1.67, 1.63, 1.56 and 1.72 respectively. The feed efficiency was best ($P \leq 0.05$) for 3.0 per cent Azolla (T3) fed group and it was statistically similar to control (T1) and 1.5 per cent Azolla fed (T2) groups. The birds fed with 4.5 per cent Azolla (T4) had significantly inferior feed efficiency than 3.0 per cent Azolla fed group (T3).

The mean cumulative feed efficiency of birds calculated for the treatments T1, T2, T3 and T4 were 1.54, 1.56, 1.60 and 1.59, respectively up to four weeks of age and 1.68, 1.71, 1.75 and 1.74 respectively up to six week of age. The statistical analysis of data on cumulative feed efficiency up to four and six weeks of age revealed no significant differences among the treatment groups.

The cumulative feed efficiency up to four weeks of age due to Azolla inclusion at the levels of 1.5, 3.0 and 4.5 per cent levels was more to the tune of 0.02, 0.06 and 0.05 than the control, whereas the cumulative feed efficiency up to six weeks of age was more only to the extent of 0.03, 0.07 and 0.06, respectively than the control. It shows that though, feed efficiency was numerically more with Azolla supplemented groups, the differences were negligible.

Similar results were reported by Parthasarathy *et al.* (2002). According to them, feed and protein efficiency ratio in broilers were in the same order as in basal and 5 per cent Azolla diets, replacing 2.6 per cent wheat bran and 2.4 per cent fish meal. Similarly, Raseena (2006) reported that the cumulative mean feed efficiencies in quail layers based on per dozen eggs and per kg egg mass for the different dietary groups fed with 0, 1.5, 3 and 4.5 per cent Azolla showed no significant difference among the treatments.

On the other hand, Ardakani *et al.* (1996) observed better feed efficiency in broiler birds fed with 6 per cent fresh Azolla compared to control birds. However, Khatun *et al.* (1999) observed that feed efficiency reduced significantly when laying hens were fed with Azolla diets formulated on a total protein and total amino acid basis. Reports of Basak *et al.* (2002) and Parthasarathy *et al.* (2002) also indicated that higher levels of Azolla (10 to 20 %) significantly reduced feed efficiency in broiler chicken.

Buckingham *et al.* (1978) reported that higher levels of acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin in Azolla could be the reason for the poor feed utilization in monogatric animals. However, in the present study maximum level of Azolla incorporation was 4.5 per cent.

Based on the results of this experiment, it is clear that the dried Azolla could be used in broiler chicken ration up to 4.5 per cent level without any adverse effect on feed efficiency.

5.7 PROCESSING YIELDS AND LOSSES

The data on processing yields and losses presented in Table 12 revealed that all parameters studied except giblet yield and blood loss did not differ significantly between the treatments.

Mean dressed yield and ready to cook yield of T1, T2, T3 and T4 were 91.85, 92.43, 92.14 and 92.10 per cent and 74.01, 72.74, 72.76 and 75.60 per cent, respectively. The statistical analysis of data on mean per cent dressed yield and ready-to-cook yield did not show any significant difference among treatment groups. On the other hand, Basak *et al.* (2002) reported higher dressing per cent in broiler birds fed with 5 per cent Azolla.

The mean giblet yields were 4.13, 4.08, 3.81 and 4.35 per cent respectively for T1, T2, T3 and T4 groups. The birds fed with 4.5 per cent Azolla (T4) recorded significantly higher giblet yield ($P \leq 0.05$) than the birds fed 3.0 per cent Azolla (T3). The giblet yield obtained for the control birds and those offered 1.5 per cent Azolla were intermediary and were statistically comparable to other two groups. Raseena (2006) found that the mean giblet weights were numerically higher for Azolla supplemented groups (1.5, 3.0 and 4.5 per cent) compared to control birds fed standard quail layer diet. But Basak *et al.* (2002) reported that giblet per cent was significantly higher in 15 per cent Azolla meal fed group followed by 10 per cent, 5 per cent and control group. In present experiment a definite trend in giblet yield due to different levels of Azolla feeding was not noticed. The reasons for this varying trend need further study and analysis.

Among per cent losses, feather loss was not influenced significantly by Azolla incorporation in feed whereas blood loss had significant influence ($P \leq 0.05$). Blood loss was significantly more with 4.5 per cent Azolla fed group and less with control birds. The blood loss values for the 1.5 and 3.0 per cent Azolla supplied groups were intermediary. In comparison to control, birds fed with Azolla at levels of 1.5, 3.0 and 4.5 per cent resulted more loss in blood to the tune of 0.39, 0.59 and 1.0 per cent, respectively. Per cent feather loss was numerically more with control birds and less with birds fed 4.5 per cent Azolla and the difference was 1.18 per cent. On screening the literature, no work could be noticed to corraborate with the above findings.

5.8 SERUM PROFILES

5.8.1 Cholesterol

An increase in the dietary proportion of Azolla resulted in a linear reduction of serum total cholesterol levels (Table 13). The mean values of serum cholesterol were 169.80, 169.71, 154.13 and 144.17 mg per dl for the groups T1, T2, T3 and T4, respectively. Azolla feeding at 3.0 and 4.5 per cent levels significantly ($P \leq 0.05$) lowered serum cholesterol values (154.13 and 144.17 mg per dl, respectively) than those of control and 1.5 per cent Azolla (T2) fed groups (169.80 and 169.71 mg per dl). The reduction in serum cholesterol in 4.5 and 3.0 per cent Azolla fed groups were 25.63 and 15.67 mg per cent, respectively than that of control.

The reduction in serum cholesterol consequent to Azolla supplementation in broiler chicken observed in this study is close in agreement with Raseena (2006) who reported that serum total cholesterol was significantly ($P \leq 0.01$) lower in 1.5, 3 and 4.5 per cent Azolla fed groups (147.79, 140.35 and 113.99 mg per dl, respectively) compared to control (167.92 mg per dl) in laying quails. Similarly, Simi (2007) concluded that dietary supplementation of turmeric at 0.4 and 0.6 per cent levels in diet of broiler chicken resulted significant reduction in serum total cholesterol to 166.02 and 157.44 mg per dl, respectively as compared to control group (175.89 mg/dl).

The reason for lower serum cholesterol as a result of Azolla feeding could be attributed to the presence of phytosterol composed of sitosterol, campesterol and stigmasterol in Azolla (Arai *et al.*, 1998). The mechanism by which plant sterols reduce cholesterol absorption was studied by Wester (2000) and reported that these sterols had been shown to reduce micelle solubilisation of cholesterol and thereby reduce serum cholesterol. Moreover, saponins in Azolla (Arai *et al.*, 1998) forms large mixed micelle by interaction with bile acids, which prevents absorption from the small intestine and increase excretion of cholesterol. The resulting accelerated metabolism of cholesterol in the liver causes its serum level to go down (Francis *et al.*, 2002).

5.8.2 Creatinine

The mean serum creatinine levels at sixth week of age presented in Table 13 indicated that the level in 4.5 per cent Azolla fed birds was statistically higher ($P \leq 0.05$) than those of control and 1.5 and 3.0 per cent Azolla offered birds. Serum creatinine values of these three groups were lower and statisticallay homogenous. The increase in serum creatinine value due to Azolla supplementation at 1.5, 3.0 and 4.5 per cent levels was to the tune of 0.20, 0.28 and 0.70 mg per dl, respectively.

On reviewing the literature, it seems that the effect of Azolla supplementation on serum creatinine levels in chicken has not been reported. In another study, Uko and Kamalu (2006) fed 75, 150 or 225 g autoclaved neem seed kernel (AFK/kg) and 75 g raw neem seed kernel in chicks for 35 days and reported that chicks fed on the reference or test diets did not have any influence on serum creatinine levels. Similarly, Raseena (2006) also reported that serum creatinine values were not significantly influnced by the inclusion of Azolla at levels of 1.5, 3.0 and 4.5 per cent in Japanese quail ration.

5.8.3 Protein

The mean serum total protein of broiler chicken fed with 0, 1.5, 3.0 and 4.5 per cent Azolla was 3.43, 3.09, 3.22 and 3.39 g per dl, respectively. When the magnitude of difference in mean serum protein among the treatments were tested, it was found that this trait was not differed statistically. This confirms that protein of Azolla was efficiently utilized in broiler ration without any adverse effect in body weight and weight gain. These results were in agreement with the report of Raseena (2006) who found that serum protein values were not influenced due to different levels of inclusion of Azolla (1.5, 3.0 and 4.5 per cent) in Japanese quail ration. Similarly, Lonkar (2006) reported that supplementation of garlic powder and neem seed cake and their combination did not influence the serum total protein values in six week old broiler chicken.

5.8.4 Uric acid

Uric acid is the major end product of protein metabolism in birds. The serum uric acid level of 1.5 per cent Azolla fed birds was same as that of control (2.71 mg per dl). However, enhancing the level of Azolla to 3.0 and 4.5 per cent levels resulted in an increase of 0.36 and 0.46 mg per dl of serum uric acid, respectively from the control. Statistical analysis revealed that there were no significant differences with respect to mean values of serum uric acid (2.71, 2.71, 3.07 and 3.17 mg per dl in T1, T2, T3 and T4, respectively) among different treatments. It could therefore be stated that protein of Azolla was of good quality, which lead to better protein utilization and consequently resulted in reduced uric acid excretion. This result was comparable with the findings of Raseena (2006) who also observed that serum uric acid values were not influenced due to inclusion of Azolla at levels of 1.5, 3.0 and 4.5 per cent in Japanese quail rations.

Buckingham *et al.* (1978) opined that despite of higher per cent of purine in Azolla, the supplementation of it did not reflect in the serum uric acid level up to 4.5 per cent level.

5.9 BREAST AND THIGH MEAT TOTAL CHOLESTEROL

The mean breast and thigh meat total cholesterol as influenced by feeding of varying levels of Azolla presented in Table 14 indicated that as the level of Azolla increased, there was a linear reduction in meat cholesterol which was significantly different between treatments. The maximum reduction of breast meat cholesterol was achieved in T4 followed by T3 and T2 in ascending order.

The highest breast meat cholesterol was observed in the control (74.45 mg per dl) followed by 1.5 per cent (70.16 mg per dl) and 3.0 per cent Azolla fed groups (63.55 mg per dl) at sixth week of age, whereas the lowest value was noted in 4.5 per cent Azolla fed group (53.44 mg per dl).

The trend of result in thigh meat cholesterol was comparatively similar to that of breast meat cholesterol. Statistical analysis of data revealed significant ($P \leq 0.05$) differences among the treatments. The highest thigh meat cholesterol was observed in the control (T1) and 1.5 per cent Azolla fed (T2) group (122.75 and 122.78 mg per dl, respectively) with no significant difference between them, whereas the lowest ($P \leq 0.05$) value was noted in the 4.5 per cent Azolla (T4) fed group (105.54 mg per dl). The 3.0 per cent Azolla fed group (T3) had intermediary thigh meat cholesterol level of 116.84 mg per dl and was statistically different from both the groups. Lonkar (2006) studied the effect of garlic (*Allium sativum*) powder (GP) and neem (*Azadirachta indica*) seed cake (NSC) on cholesterol content in broiler chicken and reported that the supplementation of GP and NSC either alone or in combination (0.5 and 1.0 per cent GP and 1.0 and 2.0 per cent NSC) significantly ($P \leq 0.05$) reduced the breast and thigh meat total cholesterol in broiler chicken. Similarly, supplementation of turmeric at 0.4 per cent resulted in slight reduction of thigh meat cholesterol in broilers (Simi, 2007). Plant stanol esters effectively restrict the absorption of both dietary and biliary cholesterol causing specific reductions in serum total and LDL cholesterol levels thereby reducing the cholesterol level in meat (Wester, 2000).

Generally, the cholesterol concentration is much higher in the thigh meat than that of breast meat. A possible explanation is that cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast muscle. Also, thigh muscles have a much greater content of slow-twitch fibers than the breast muscles. Slow-twitch fibers have more mitochondria, their mitochondria are bigger and the metabolic rate much faster in comparison to fast-twitch fibers. Slow-twitch sarcoplasmic reticulums are found to contain two to three times as much cholesterol as fast-twitch sarcoplasmic reticulums (Bloch, 1991).

5.10 LIVABILITY

The livability of birds (Table 15) in all the four treatments were similar up to two weeks of age. However, at the end of the experiment birds in 3.0 per cent and 4.5 per cent Azolla fed groups had slightly lower livability.

In general, livability percentage was satisfactory in Azolla fed birds compared to control (Table 15). These results are comparable with the findings of Parthasarathy *et al.* (2002), who reported that the feeding of Azolla up to a level of 20 per cent did not have any adverse effect on the livability of broiler chicken. Similarly, Basak *et al.* (2002) and Alalade and Iyayi (2006) also reported that Azolla meal up to 15 per cent level in the diet of broiler and egg type chicks did not have any adverse effect on the livability of the birds.

Better survivability in Azolla fed groups can be attributed to the presence of saponins and carotenoids in Azolla (Arai *et al.*, 1998). Saponins reportedly induce production of cytokines such as interleukins and interferons that might mediate their immunostimulant effects (Francis *et al.*, 2002). Increased level of carotenoids in Azolla, which is an immunostimulant also increases survivability.

5.11 ECONOMICS

The economics of broiler production as influenced by dietary inclusion of dried Azolla was worked out and are presented in Table 16. The inclusion of Azolla by replacing conventional feed ingredients was reflected in the feed cost. There was a linear reduction in the feed cost with increasing levels of Azolla.

The mean body weights at sixth week of age for the dietary groups T1, T2, T3 and T4 were 2182.15, 2108.72, 2089.81 and 2103.51 g respectively. The feed cost incurred for obtaining the above body weights in the dietary groups T1 to T4 were Rs. 42.16, 41.61, 40.84 and 41.45 respectively. The total cost of production including feed cost, chick cost and miscellaneous cost was worked out to Rs.60.66, 60.11, 59.34 and 59.95 and the return from sale of each bird was found to rupees 98.20, 94.89, 94.04 and 94.66 for the groups T1, T2, T3 and T4, respectively.

A critical analysis of the cost benefit ratio revealed that the net profit per kg body weight was more in the birds fed with the control diet. In comparison to the control group, net profit per bird was lesser to the tune of rupees 2.76, 2.84 and 2.83 for the 1.5, 3.0 and 4.5 per cent Azolla fed groups respectively. If the net profit per kg broiler chicken was worked out, the corresponding reduction in profit for the above groups was to a tune of 0.70, 0.58 and 0.69 rupees respectively from that of control. Even though the feed cost per kg diet and the cumulative feeding cost were lower in Azolla fed groups, the margin of return over feed cost was lower in all these groups as compared to the control group. This was due to comparatively lower body weight noticed in these birds at sixth week of age.

All the Azolla supplemented groups were economically less beneficial in the present trial. Contrary to this, Parthasarathy *et al.* (2001a) reported that incorporation of Azolla as a feed ingredient at 5 per cent level in broiler rations could provide promising economic returns. Similarly, Basak *et al.* (2002) also reported that Azolla meal is an unconventional feed ingredient at lower price and may be used up to 5 per cent level in broiler diet to reduce feed cost. Among the Azolla supplemented groups the net profit per kg body weight was more (Rs.16.99) in birds fed with 3.0 per cent Azolla (T3) followed by treatments T4 (Rs. 16.88) and T2 (Rs. 16.87). However, the differences between the treatments were meagre.

Numerically higher body weight at the end of six weeks of age was the only factor that caused higher profit margin in the control group over the other Azolla supplemented groups.

Significantly lower breast and thigh meat cholesterol obtained by feeding varying levels of Azolla outclass the lower profit margin calculated for these groups.

SUMMARY

6. SUMMARY

An experiment was carried out at the Department of Poultry Science, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, to study the effect of dietary inclusion of dried Azolla on growth performance of broiler chicken. The study was conducted for a period of six weeks with one hundred and sixty, day-old commercial broiler chicks (Vencob). The chicks were wing banded, weighed individually and randomly allotted to four dietary groups T1, T2, T3 and T4. Each group comprised of four replicates of ten birds each. The group T1 was fed a control ration formulated as per the BIS specifications (1992). Sun dried and ground Azolla at 1.5, 3.0 and 4.5 per cent levels were incorporated in the rations for T2, T3 and T4,respectively. All the rations were made isocaloric and isonitrogenous.

Standard managemental practices were followed throughout the experimental period. Feed and water were provided *ad libitum*. The birds were provided with starter ration from 0 to 4 weeks of age and thereafter finisher ration during fifth and sixth week of age.

The production performance of broilers was evaluated for a period of six weeks. The weekly body weight was recorded individually and feed consumption was recorded replicate wise. From these data, weekly weight gain and feed conversion ratios were calculated.

At the end of sixth week two birds from each replicate were sacrificed to study the mean per cent dressed yield, eviscerated yield, per cent weight of giblet and ready to cook yield. The meat from breast and thigh were collected from each carcass and the total cholesterol was estimated. Blood samples were collected at the time of slaughter for the estimation of serum total cholesterol, serum creatinine, serum total protein and serum uric acid. Economics over feed cost was calculated based on the prevailing cost of feed ingredients. The sale price of broiler chicken based on live weight was used for evaluating the economics of incorporation of Azolla in broiler diets.

Based on the results obtained in the study, the following conclusions were made,

1. The overall mean maximum and minimum temperatures (°C) and relative humidity (%) in the forenoon and in the afternoon inside the experimental house during the experimental period were 29.90, 24.75, 79.07 and 65.78, respectively.

2. The mean body weight of broilers at six week of age in dietary groups T1, T2, T3 and T4 were 2182.15, 2108.72, 2089.81 and 2103.51 g respectively and statistical analysis of this data revealed no significant difference among the treatments.

3. The mean body weight gain of broilers at sixth week of age in dietary groups T1, T2, T3 and T4 were 2123.03, 2123.03, 2066.03 and 2039.78 g, respectively and statistical analysis revealed no significant difference among the treatments.

4. The mean cumulative feed intake of broiler chicken from 0 to 6 weeks of age were 3676.65, 3655.18, 3659.05 and 3721.59 g in dietary groups T1, T2, T3 and T4 respectively. Statistical interpretation of 0 to 4 and 0 to 6 weeks cumulative feed intake data revealed that this trait was not influenced by Azolla addition at varying levels in the feed.

5. The mean cumulative feed conversion ratio (FCR) up to six weeks recorded for the dietary groups T1, T2, T3 and T4 were 1.68, 1.71, 1.75 and 1.74, respectively. The mean feed conversion ratio did not reveal statistical difference among the dietary groups.

6. Dietary supplementation of dried Azolla did not influence the mean per cent dressed and ready-to-cook yield among the dietary groups and the mean values in T1, T2, T3 and T4 were 91.85, 92.43, 92.14 and 92.10 per cent and 74.01, 72.74, 72.76 and 75.60 per cent, respectively.

7. The mean per cent giblet yield in dietary groups T1, T2, T3 and T4 were 4.13, 4.08, 3.81 and 4.35 respectively. The birds fed with 4.5 per cent Azolla (T4) recorded significantly higher giblet yield ($P \leq 0.05$) than control and other Azolla supplemented treatments.

8. The blood loss in the group T4 fed with 4.5 per cent Azolla (3.84 per cent) was significantly more than that of control group T1 (2.84 per cent), whereas the feather loss and total loss were not influenced by the dietary supplementation of dried Azolla.

9. The supplementation of Azolla at 3.0 and 4.5 per cent levels (T3 and T4) resulted significant ($P \leq 0.05$) reduction in serum cholesterol level to 154.13 and 144.17 mg per dl, respectively when compared to the mean value of 169.80 mg per dl, in the control group.

10. The mean serum creatinine levels (mg per dl) estimated for the treatments viz., T1, T2, T3 and T4 were 2.41, 2.61, 2.69 and 3.11, respectively. The birds fed on 4.5 per cent Azolla (T4) had significantly higher serum creatinine value than those of other treatment groups.

11. On the other hand, serum total protein and uric acid were not influenced due to Azolla supplementation in broiler ration.

12. The inclusion of Azolla at the levels of 1.5, 3.0 and 4.5 per cent in broiler rations significantly ($P \leq 0.05$) reduced the breast meat cholesterol in broilers.

13. The Azolla feeding at 3.0 and 4.5 per cent levels significantly reduced the thigh meat cholesterol in broilers.

14. The livability of birds in all four treatments were comparatively similar up to two weeks of age. However, cumulative livability of birds in 3.0 and 4.5 per cent Azolla supplemented groups showed 5 and 2.5 per cent lower livability than control and 1.5 per cent Azolla fed group.

15. The cost benefit analysis revealed that the net profit per bird was the highest in group fed control ration (Rs.17.57) followed by T3 (Rs.16.99), T4 (16.88) and the lowest being in the group T2 fed 1.5 per cent Azolla (Rs.16.87).

Based on the results obtained in the present study, it could be concluded that the dried Azolla can be included in broiler feed up to 4.5 per cent level. Even though margin of profit is slightly low in 4.5 per cent Azolla added group, the merit of low cholesterol meat by feeding Azolla is advantageous. The broiler meat having less cholesterol can be marketed at higher unit price and it can offset the lower body weight of broiler chicken resulted from feeding Azolla.

Table 17. Summary of results of the study to assess the influence of dried Azolla on the performance and cholesterol content of meat and serum of broiler chicken.

Parameter	Period in weeks	Dietary treatments			
		T1	**T2**	**T3**	**T4**
Live body weight, g	6	2182.15	2108.72	2089.81	2103.51
Cumulative body weight gain (g)	0 - 4	1156.35	1146.61	1138.78	1107.99
	0 - 6	2123.03	2123.03	2066.03	2039.78
Cumulative feed intake (g)	0 - 4	1803.20	1806.38	1821.38	1793.09
	0 - 6	3676.65	3655.18	3659.05	3721.59
Cumulative feed efficiency (kg feed / kg weight gain)	0 - 4	1.54	1.56	1.60	1.59
	0 - 6	1.68	1.71	1.75	1.74
Serum total cholesterol (mg/dl)	6	169.80 [b]	169.71 [b]	154.13 [a]	144.17 [a]
Serum creatinine (mg/dl)	6	2.41 [a]	2.61 [a]	2.69 [a]	3.11 [b]
Serum total protein (mg/dl)	6	3.43	3.09	3.22	3.39
Serum uric acid (mg/dl)	6	2.71	2.71	3.07	3.17
Breast meat total cholesterol (mg/dl)	6	74.45 [d]	70.16 [c]	63.55 [b]	53.44 [a]
Thigh meat total cholesterol (mg/dl)	6	122.75 [c]	122.78 [c]	116.84 [b]	105.54 [a]

Means bearing the different superscript within the same row differ significantly ($P \leq 0.05$)

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EFFECT OF DIETARY INCLUSION OF AZOLLA (*Azolla pinnata*) ON PRODUCTION PERFORMANCE OF BROILER CHICKEN

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Master of Veterinary Science

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ABSTRACT

An experiment was carried out at the Department of Poultry Science, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, to investigate the effect of dietary inclusion of sun dried and ground Azolla (*Azolla pinnata*) on growth performance of broiler chicken.

The study was conducted for a period of six weeks with one hundred and sixty, day-old commercial broiler chicks. The chicks were divided into four different treatments with four replicates of ten chicks each. The T1 was control with standard broiler ration and dried Azolla powder was supplemented in the basal diet at 1.5 per cent (T2), 3.0 per cent (T3) and 4.5 per cent (T4). The rations were made isocaloric and isonitrogenous. Chicks were reared under standard managemental conditions up to six weeks of age. The broiler starter ration was fed from 0 to 4 weeks of age and thereafter finisher ration during fifth and sixth week of age.

Results of the present study revealed that supplementation of dried Azolla did not influence final body weight of broilers. The cumulative body weight gain up to sixth week of age did not reveal any significant difference among treatments. However, there was numerical reduction in both body weight and weight gain due to Azolla supplementation. The cumulative feed intake up to sixth week of age did not reveal any significant difference among treatments. But numerically lower feed consumption was recorded in 1.5 per cent (T2) and 3.0 per cent (T3) Azolla supplemented groups. The cumulative feed efficiency up to six week of age revealed no significant difference among the treatment groups. Although feed efficiency was numerically more with Azolla supplemented groups the differences were negligible. The per cent dressed yield, eviscerated yield, ready-to-cook yield, feather loss and total loss in broilers were not influenced by dietary supplementation of dried Azolla. The per cent giblet yield of birds fed with 4.5 per cent Azolla (T4) was significantly higher ($P \leq 0.05$) than control and other treatments. Giblet yield was low ($P \leq 0.05$) with 3.0 per cent Azolla supplemented group. The dietary supplementation of dried Azolla at 1.5 per cent level numerically reduced the serum total cholesterol level, while at 3.0 and 4.5 per cent levels (T3 and T4) there were

significant ($P \leq 0.05$) reductions in serum total cholesterol. The dietary supplementation of dried Azolla at 4.5 (T4) per cent had significantly higher ($P \leq 0.05$) serum creatinine value than other treatment groups. The supplementation of Azolla at 1.5, 3.0 and 4.5 per cent levels significantly ($P \leq 0.05$) reduced the breast meat cholesterol in broilers. The supplementation of Azolla at 3.0 and 4.5 per cent levels significantly reduced the thigh meat cholesterol in broilers than control and 1.5 per cent Azolla fed groups. The livability of birds in all four treatments were comparatively similar up to two weeks of age. However, at the end of experiment birds in 3.0 and 4.5 per cent Azolla supplemented groups livability per cent was less to the extent of 5 and 2.5 per cent respectively than control and 1.5 per cent Azolla fed group.

Net profit per kg body weight was slightly lowered as a result of inclusion of dried Azolla at all three levels in broiler diet. The results of the present study suggest dried Azolla can be incorporated in broiler rations up to 4.5 per cent without affecting the economic traits. Moreover, dietary inclusion of dried Azolla in broiler rations is beneficial for substantial reduction in the cholesterol content of poultry meat.