STUDIES ON THE VALUE OF *AZOLLA PINNATA* ON LAYER PERFORMANCE

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CERTIFICATE

This is to Certify that the thesis entitled **STUDIES ON THE VALUE OF *AZOLLA PINNATA* ON LAYER PERFORMANCE** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN ANIMAL NUTRITION TO THE TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY, MADRAS** is a record of bonafide research work carried out by **Thiru. B. CHANDRABOSE** under my supervision and guidance and that no part of this thesis has been submitted for the award of any degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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STUDIES ON THE VALUE OF *AZOLLA PINNATA* ON LAYER PERFORMANCE

ABSTRACT

A study was conducted to assess the feeding value of *Azolla* (*Azolla pinnata*) in layer birds from 0 - 40 weeks of age. The metabolism trial and a nitrogen balance trial were also conducted at 40 weeks of age.

Four samples of *Azolla pinnata* were collected from four different locations in Tamilnadu and analysed for chemical composition. The fresh samples of *Azolla pinnata* had a mean dry matter content of 12.16 ± 1.21 per cent. The sundried samples of *Azolla* contained 25.46 ± 0.69 per cent crude protein, 1.88 ± 1.11 per cent ether extract, 12.46 ± 0.55 per cent crude fibre, 15.06 ± 1.03 per cent total ash and 6.80 ± 0.06 per cent insoluble ash. The overall mean values for NDF, ADF, cellulose, hemicellulose and lignin were 42.39, 27.94, 12.44, 14.62 and 6.93 per cent respectively. Among the minerals analysed, sundried *Azolla* contained 1.48 ± 0.18 per cent calcium, 0.64 ± 0.12 per cent phosphorus and 2.56 ± 0.54 per cent potassium. Among the trace elements, the level of iron was high with 1321 mg/kg. The lead content was very low with a mean of only 0.03 mg/kg. The amino acids arginine, leucine, valine and isoleucine were high. While methionine and cystine were deficient in *Azolla* protein.

Azolla contained an apparent metabolizable energy value of 1350 Kcal/kg while the true metabolizable energy was 1659 Kcal/kg. The two feeding experiments conducted in layer chicks from 0-8 weeks of age indicated that inclusion of *Azolla*, replacing a protein mix of groundnut cake and wheat bran (50:50) depressed the

growth with marginal decrease in feed and protein efficiencies. The growth depression was more severe at 10 and 15 per cent levels.

The performance of growers on rations containing *Azolla* at 0, 5, 10 and 15 per cent levels was assessed in a separate trial from 9-20 weeks of age. The weight gain at 20 weeks of age on 0, 5, 10 and 15 per cent *Azolla* diets was 698.2, 657.3, 710.7 and 692.3 g respectively. The feed intake was marginally high resulting in marginal decrease in feed efficiency on rations containing *Azolla*. The data is suggestive that *Azolla* can be included even at 15 per cent level in grower rations.

Two separate feeding trials were conducted in layers from 20-40 weeks of age. In the first trial, *Azolla* was included at 0, 5, 10 and 15 per cent levels and the weight gain at 40 weeks of age was 393.0, 371.6, 364.5 and 344.8 g respectively. The egg production in the control group was 109 and 110 eggs in the 15 per cent group.

The feed efficiency and the various parameters on egg quality studies except yolk colour did not vary among the treatments. The yolk colour as expressed in terms of Roche fan colour score varied from 6.22 in the basal group to 7.41 in the 15 per cent *Azolla* group.

In the second trial dried *Azolla* was included at 0, 15, 20 and 25 per cent levels. The weight gain in the control group of birds was 405 which decreased progressively to 396.6, 351.2 and 316.0 g with increasing levels of *Azolla* at 15, 20 and 25 per cent. The differences were significant between the treatments at 5 per cent level. The number of eggs laid and feed efficiency declined marginally with increasing levels of *Azolla* as in the earlier experiment. The egg quality parameters studied were similar to the observations made earlier.

The histopathological examination of internal organs revealed mild congestion, diffused vascular degenerative changes in hepatocytes and mild infiltration of mononuclear cells in the lamina propria of small intestine.

On the basis of the observations made in the various experiments, it is concluded that addition of *Azolla* at 5 per cent level in the chick mash may not be advantageous, while in grower and layer rations it may be included at 15 per cent level.

ABBREVIATIONS USED

AAS	-	Atomic Absorption spectrophotometer
ADF	-	Acid detergent fibre
AIA	-	Acid Insoluble ash
AME	-	Apparent metabolizable energy
AOAC	-	Association of Analytical chemists
BGAP	-	Blue green algae project
CF	-	Crude fibre
CP	-	Crude protein
DCP	-	Digestible crude protein
DM	-	Dry matter
DMB	-	Dry matter basis
EE	-	Ether extraction
HPLC	-	High performance liquid chromatography
ME	-	Metabolizable energy
NFE	-	Nitrogen free extract
NDF	-	Neutral detergent fibre
SBM	-	Soybean meal
TA	-	Total ash
TDN	-	Total digestible nutrients
TME	-	True metabolizable energy
TNAU	-	Tamilnadu Agricultural University331
TRRI	-	Tamilnadu Rice Research Institute
WLH	-	White Leghorn331

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Introduction

CHAPTER 1

INTRODUCTION

Poultry production, an integral part of rural agriculture but reaching industrial proportions in recent years, is an important source of animal protein in meeting the demands of growing human population. Over the last decade poultry population has grown spectacularly throughout the world showing an increase of 23 per cent in developed countries and 76 per cent in developing countries. However, it is in the fareast that this progress has been most notable; on an average 90 per cent, in India production has increased six fold in ten years (PIYB, 1994).

In India there were 101 million poultry in 1980 which increased to 122 million in 1985 and further to 154 million in 1990. For the present, the estimated population of layers alone is 170 million. Similarly the egg production had also increased over the years, from 12500 million in 1980 to 23300 million in 1990, while the estimate is 28130 million eggs during 1995 (Anon, 1994). Thus the poultry population and egg production have grown by about 170 and 225 per cent respectively during the period from 1980 to 1995. The enhanced production is mainly due to the progress made through research in breeding, nutrition, health cover and management.

The increase in poultry population had seen a concurrent growth in the production of concentrate feed. The annual poultry feed production was a meagre 2.5, 3.0, 4.7 and 5.3 million tonnes during 1980, 1985, 1988 and 1994 respectively, while the estimated requirement in 2000 AD is 10 million tonnes (Singh *et al.,* 1991). In another estimate Bessei (1994) had calculated a requirement of 9.5 million tonnes of feed for an estimated population of 145 million layers and 750 million broilers.

However, there is a shortage of grains and other conventional feed ingredients useful for poultry due to preferential use of grains for human consumption, and shrinking aerablé land due to urbanization. Thus there have been a continued search over the past couple of decades for newer non-traditional feeds. There are a large number of non-traditional feeds which are locally available and such feedstuffs include crop residues, agro-industrial by-products, forest by-products, animal wastes including slaughter house by-products and aquatic/ marine by-products.

Several agricultural by-products like spent brewers grain (Punj, 1988), cashew apple meal (Kadirvel *et al.,* 1994) and industrial by-products like dried yeast sludge (Senthil Kumar, 1994) have been identified as unconventional feed stuffs for poultry. The use of such non-conventional feeds is also limited because of their poor nutritive value, seasonal availability, problems of storage and processing and due to increased presence of anti-nutritional factors in such feeds. Excellent reviews on the availability and utilization of crop residues (Verma, 1988), non-conventional feeds for ruminants and non-ruminants (Punj, 1988; Gupta, 1988; Boda, 1990; El Boushy and van der Pòel, 1994) are available.

The aquatic plants found in the sea, lakes, ponds and rivers have also been examined for their utility as livestock feed. Some of the common aquatic plants viz. *Myriophyllum, Coratophyllum, Pontamogeton, Nymphaea, Hydrilla, Salivinia, Ipomaea aquatica, Caratoptens* species, *Nekumbium, Speciosum* and *Eichomia Crassiper* (Ranjhan, 1990) have also been examined for their utility as livestock feed. Ali and Leeson (1994) reported a total harvest of 24 million metric tonnes of aquatic plants in the world. Duck weed, *Hydrilla* and water hyacinth were evaluated for their nutrient quality (Muztar *et al.,* 1976; Alcantera and Querubin, 1985; Boyd, 1968).

Preliminary investigations have established the usefulness of *Hydrilla* and *Elodea* in layer and chick rations (McDowell *et al.*, 1990; Lizama *et al.*, 1988).

Among the aquatic plants, *Azolla* is an important source of protein due to the occurrence of both photosynthesis and nitrogen fixation in their leaves. *Azolla* is rich in amino acids, minerals (Kannaiyan, 1985; Sanginga *et al.*, 1989) vitamins, carotenoids and xanthophylls (Dewanji, 1993). Unlike other lower plants, *Azolla* is capable of quick multiplication, free from any toxicants and above all easy to cultivate as well. The production of *Azolla* has been estimated at 5.5 thousand metric tonnes (Ali and Leeson, 1994) however, much work has not been carried out regarding the use of *Azolla* in layers and hence the present study with the following objectives :

1) to determine the proximate composition and mineral content of *Azolla pinnata*
2) to study the effect of feeding *Azolla* containing rations on growth and feed efficiency in chicks from 0-8 weeks of age.
3) to determine the metabolizable energy content and nitrogen retention in layers.
4) to study the effect of inclusion of *Azolla* in grower rations on their growth and feed efficiency from 9-20 weeks of age.
5) to assess the value of feeding *Azolla* based diets on layer performance and egg quality from 21-40 weeks of age.
6) to assess the economics of using *Azolla* as a possible non-conventional feed in layer

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

2.1 Aquatic Plants - General Nutritional Characteristics

The aquatic plants are highly productive crop requiring no tillage, fertilizer, seed or cultivation. But, unfortunately they burden water ways affecting irrigation, disease and insect control, fish production and water quality. However there is scope for advantageous use of aquatic plants as animal feed, for fuel production, as soil additives and waste water treatment.

Fresh aquatic weeds usually have too much moisture (90-95 per cent) to be efficient feedstuff and most aquatic weeds may contain as much as 95 per cent moisture. The high moisture content and consequent bulkiness make aquatic weed cumbersome, expensive to transport, and impossible to store without mould forming. The protein content on dry matter basis may vary between 10-25 per cent which is equivalent to or higher than that of forages. The leafy portion of some aquatic plants such as duck weed and water hyacinth may contain 25-35 per cent protein (DMB) which is exceptionally high.

Dewanji (1993) estimated the chemical composition and amino acid profile of leaf protein extracted from five aquatic weeds including *Azolla pinnata*. The results indicated that nitrogen content of leaf protein ranged from 6.1 to 8.7 per cent. He concluded that leaf protein of uniform composition could be extracted from aquatic plants. The essential amino acid levels of proteins compared favourably with human and chick requirement. The amino acid profile may also be similar to that of forages. The amino acids lysine and methionine which are generally considered as limiting in plant proteins are often lower than in terrestrial forages. But Taylor and Robbins (1968) had observed that lysine content in water hyacinth is equal to that found in milk, suggesting that amino acid profile in some of these aquatic plants may be more desirable. The fibre content of aquatic weeds may be highly variable. The submerged weeds may be low in fibre as compared to the floating and emergent weeds while some of the tall weeds like reeds and cocktails may be having extreme amount of fibre.

The mineral content in freshly harvested aquatic plants may vary depending on the plant type and on the condition of the water way. The ash content of submerged plants and floating plant with long roots covered in sand, silt and encrusted carbonates may be as high as 60 per cent which would make them unsuitable for consideration as animal feed. The mineral content within the plant may also vary depending upon the level of dissolved minerals in the water way which might be limiting in their use as animal feed.

Apart from the nutrient constraints referred above, aquatic plants may contain traces of pesticides. Water ways linked to an agricultural area that received regular application of insecticides may create problem of residues in water ways. Similarly canals and water ways which were excessively polluted with animal and human wastes may contain pathogenic bacteria.

Water plants are good sources of xanthophyll and carotene. Limcango (1989) reported a concentration of 500-600 mg of xanthophyll per kilo of feed. Similarly Dewanji (1993) reported the presence of 462.5 to 674.7 µg of ß-carotene per gram of feed. The water plants if processed properly, would be good pigmenting agents.

The aquatic plants that are potential livestock feeds fall under five different categories namely floating, submerged, emergent, algae and sea weeds.

2.2 Floating species

2.2.1 Duck Weeds

These are tiny, free floating plants having world wide distribution. The crude protein content (DMB) of duck weeds obtained from natural sources range from 10 - 20 per cent (Tan, 1970). Duck weeds are consumed by domestic and wild fowl and their nutritional value recognised (Lautner and Muller, 1954).

Truax *et al.,* (1972) had reported that dehydrated duck weed can substitute for dried alfalfa meal upto 5 per cent of mixed poultry feeds resulting in superior weight gain in chicks. Similarly Johri and Sharma (1979) reported beneficial response on inclusion of duck weed at a level of 10 per cent in chick starter or broiler diets and 15 per cent in layer rations. However Muztar *et al.,* (1976) and Haustein *et al.,* (1992) reported a 10 per cent decrease in growth of broilers fed 10 per cent duck weed based diets. Further the same group of workers reported a linear decrease in live weight of broilers fed graded levels of duck weed upto 400 g/kg of the ration.

2.2.2 Water hyacinth

Sun dried water hyacinth is a fairly good source of crude protein (17 per cent) and amino acid lysine (1.2 per cent) but it is high in fibre and ash (Boyd, 1974). It can be used as a feed for ruminants and pigs, but its high potash and chloride may cause problems of digestive disorders.

Smetana (1968) had recommended the use of water hyacinth as a green feed for domestic fowl and ducks, while Khalil *et al.,* (1975) recommended the inclusion of water hyacinth meal in the diet of young chicks as a substitute for corn.

Soesiawaningrini *et al.*, (1979) fed ground and dried water hyacinth in different proportions with other commercial feed to one week old ducklings upto 7.5 per cent without any adverse effect on weight gain and feed efficiency. But Solly *et al.,* (1984) reported adverse effect on growth and feed conversion of ducklings when water hyacinth meal was used in the ration. He also opined that it is a poor source of vitamins.

2.3 Submerged species

2.3.1 *Elodea*

According to Maurice *et al.,* (1984) *Elodea* contains 13-15 per cent CP and 11 per cent fat. The amino acids glycine, lysine, threonine and valine are reported to be higher than in maize. It is a good source of xanthophyll as well.

Sun dried *Elodea* was included at 5 per cent in the diet of fast growing broilers without any adverse effect on growth or feed efficiency (Maurice *et al.,* 1984). However, Lizama *et al.,* (1988) observed depressed growth, poor feed efficiency and lowered carcass dressing percentage with higher levels of *Elodea*. In laying hens *Elodea* was used beneficially at 7.5 per cent of the diet with improved yolk pigmentation (McDowell *et al.*, 1990).

2.3.2 *Hydrilla*

Hydrilla is found in all tropical and subtropical countries, and on dry matter basis contains around 16.5 per cent crude protein, 3.5 per cent fat and 9.35 per cent crude fibre. The amino acids lysine, threonine and valine are higher than maize and other grains (Lizama *et al.*, 1988).

Hydrilla at a level of 5 per cent in the diet reduced broiler performance (Lizama *et al.*, 1988) which was attributed to higher level of ash in hydrilla. But McDowell *et al.*, (1990) observed satisfactory performance of layers, when sundried hydrilla was incorporated in layer ration at 7.5 per cent apart from improving yolk pigmentation.

2.4 Emergent species

2.4.1 Algae

Sundried algae is rich in protein (51-63 per cent) but low in fibre (1-6 per cent) and ash (6-11 per cent). Algae contains an amino acid profile similar to that of soyabean meal and it is a rich source of xanthophyll, ß carotene, thiamine, riboflavin, pyridoxine, vit B12 and vit C (Clement *et al.*, 1967, Paoletti *et al.*, 1971, Anderson *et al.*, 1991).

According to Grau and Klein (1957) birds can tolerate upto 20 per cent of aluminium free algae meal but not beyond that level which depressed growth. Young chicks and broilers performed well on diets containing 10 per cent algae meal (Combs, 1952; Lipstein and Hurwitz, 1980). Similarly, Lipstein *et al.,* (1980) observed satisfactory performance of layers and quail breeders on diets containing upto 12 per cent algae meal. But McDowell and Leveille (1963) reported poorer performance of chicks and rats fed diets supplemented with algae meal even at low levels of inclusion.

The addition of blue-green algae at 1 per cent level of the diet helped to improve yolk pigmentation in the eggs of Japanese quails fed diet which is otherwise free of xanthophylls (Anderson *et al.,* 1991).

2.4.2 Sea Weeds

Among the various aquatic plants, dry sea weeds are low in protein (7-9 per cent), with fibre in the range of 4-11 per cent and ash at 11-19 per cent. It is a good source of calcium. Jensen (1972) found the use of sea weeds beneficial in the diet deficient in vit A or B2.

Stephenson (1980) found a 20 - 30 per cent increase in egg production through the addition of sea weed meal at 8 per cent level in layer rations. Similarly, the inclusion of sea weed meal at 7 per cent level in the chick mash, and at 15 per cent level in a layer mash was without any ill effects (Chapman and Chapman, 1988).

Sea weed was also used to improve yolk pigmentation. According to Jensen (1963), the addition of 10-15 per cent brown sea weed meal in a carotenoid deficient layer ration improved yolk colour significantly.

2.5 *Azolla* species

2.5.1 Origin

The natural occurrence of *Azolla* in rice fields and ponds contributed to eventual recognition leading to cultivation of *Azolla* as a blue-green manure in Asia. The exact period when *Azolla* cultivation began is not found in history but its cultivation is reported as early as in 11th century in Vietnam (Dao and Tran, 1979). China and Vietnam are the two countries in the world having a long history of *Azolla* cultivation (Liu chung Chu, 1979) and *Azolla* was used as a green compost in these countries during 17th century.

Taxonomically *Azolla* belongs to the orders "*Salvinales*" and grouped with genus "*Salvinia*". It belongs to the family "*Azollaceae*". The name *Azolla* is derived from two greek words "*Azo*" means to dry and "*Allyo*" means to kill (Jaeger, 1978).

2.5.2 Distribution

Azolla is a small, floating aquatic fern found in fresh water eco-systems of temperate and tropical regions throughout the world. The habitat of *Azolla* include ponds, ditches, canals, tanks and paddy fields.

Azolla is not found in large lakes or swiftly moving waters. The distribution of six living species of *Azolla* have been reported by Moore (1969) and Lumpkin and Plucknett (1980). *Azolla filiculoides, Azolla caroliniana, Azolla mexicana* and *Azolla microphylla* are the four species under "*Euazolla*" while *Azolla pinnata* and *Azolla nilotica* are the two species under "*Rhizosperma*".

2.5.3 *Azolla* - *Anabaena* symbiotic system

Azolla contains a heterocystous blue-green algae "*Anabaena Azollae*". The symbiotic *Anabaena* in *Azolla* is associated with dorsal leaf lobes from the onset of their development and is never in direct contact with external environment. Algal symbionts are found in the cavity present in the proximal portion of dorsal lobes. *Anabaena* fixes the atmospheric nitrogen which is rapidly assimilated by *Azolla*. Thus *Azolla* derives all its total nitrogen requirement by this symbiotic relationship.

2.5.4 Multiplication of *Azolla*

Azolla has the ability of quick multiplication unlike other lower plants. Growth of *Azolla* in laboratory and nature is entirely through vegetative reproduction. However sexual reproduction for survival occurs during temporary adverse conditions.

The initial growth of *Azolla* is normally slow, which is followed by rapid growth. With increasing growth rate, the water surface gradually gets covered and the biomass yield reaches the maximum. The bio-mass doubling time for *Azolla* falls between 2-3 days (Watanabe *et al.,* 1980). The production of bio-mass varies with species and climatic conditions.

The maximum bio-mass production of *Azolla pinnata* is about one tonne dry matter/ hectare whereas the bio-mass of *Azolla filiculoides* is higher because of the stratified growth habitat of this fern (Watanabe *et al.,* 1977). Becerra *et al.,* (1990) obtained a total yield of nine tonnes per hectare in a year.

Azolla yields maximum biomass production during the months of October to February. It can be easily grown in nurseries and reports indicate the possibility of producing 40-50 kg of *Azolla* in a single harvest from an area of one cent.

2.5.5 Factors influencing growth rate of *Azolla*

The growth of *Azolla* is subjected to numerous environmental factors such as temperature, light intensity, pH, salinity, humidity, and wind (Kannaiyan 1988).

2.5.5.1 Light intensity

The solar energy to secure optimum yield from *Azolla* is 20,000 to 50,000 Lux. The growth rate increases with increasing light intensity (Peters *et al.,* 1976).

2.5.5.2 Temperature

The optimum temperature for getting rapid multiplication of *Azolla* is in the range of 20°C to 25°C. If the temperature goes beyond 25°C, the colour of the plant changes to brown and above 30°C the production drops while below 5°C the plant ceases to grow.

2.5.5.3 pH

A soil pH of 5 to 8 is considered to be optimal range for *Azolla* cultivation and according to Singh (1978) the growth of *Azolla* is poor in sandy soil.

2.5.5.4 Humidity

A relative humidity of 85 to 90 per cent is most suited for cultivation of *Azolla*. The growth is severely affected if the relative humidity is lesser than 60 and more than 90.

2.5.5.5 Wind

Wind tends to push all the fronds together on the same part of water source and such over population at a local point may inhibit the growth of *Azolla*.

2.5.6 Nutritive value of *Azolla*

2.5.6.1 Chemical Composition

2.5.6.1.1 Proximate Composition and Fibre fractions

The literature on proximate composition of *Azolla* is limited and the available information is summarised and given in Table 1.

The fresh *Azolla* contained 84.6 to 93.7 per cent moisture (Islam, 1993) with a dry matter content of 5 to 18 per cent while the sun dried *Azolla* contained 7 to 10 per cent moisture (Ali and Leeson, 1994 and Taklimi *et al.,* 1993).

The crude protein content ranged from 15.37 to 33.50 (Mohanpur, 1992; Watanabe *et al.,* 1977) and the ether extract ranged from 1.93 to 5 per cent (Querubin *et al.,* 1986 and Buckingham *et al.,* 1978).

Table 1 The per cent proximate composition of *Azolla* as reported in the literature

DM	CP	EE	CF	TA	NFE	References
Fresh *Azolla*						
4.8 - 7.7	-	-	-	-	-	Moore (1969)
6.7 - 15.4	-	-	-	-	-	Islam (1993)
Sundried *Azolla*						
-	18.75 - 33.5	-	-	-	-	Watanabe *et al.*, (1977)
-		5	-	15.5	-	Buckingham *et al.*, (1978)
-	24 - 30	3 - 3.3	9.1	10.5	-	Singh (1978)
-	29.2	-	-	33.6	-	Singh *et al.*, (1983)
90.00	15.63	2.84	13.01	15.82	52.70	Parnerkar *et al.*, (1986)
-	17.59 - 23.69	1.93 - 2.93	13.19 - 16.54	-	-	Querubin *et al.*, (1986)
-	17.25	-	-	-	-	Sanginga *et al.*, (1989)
-	15.37	-	-	32,48	-	Mohanpur (1992)
-	28.39	-	-	-	-	Majid *et al.*, (1992)
90.12	15.37	2.73	14.13	20.35	-	Tamang *et al.*, (1992)
90.00	25.33	3.01	11.60	23.59	-	Taklimi *et al.*, (1993)
93.00	15.4	2.8	14.10	20.4	47.4	Ali and Leeson (1994)

Buckingham *et al.,* (1978) had reported (DMB) ash - 15.5 per cent, acid detergent fibre - 26.6 per cent, neutral detergent fibre - 39.2 per cent, cellulose - 15.2 per cent and lignin - 9.3 per cent, while Singh *et al.,* (1983) recorded 41 per cent neutral detergent fibre and 33.6 per cent total ash which included 17.5 per cent acid insoluble ash.

Tamang *et al.,* (1992) estimated 67.54 per cent neutral detergent fibre, 51.96 per cent acid detergent fibre, 15.61 per cent hemicellulose, 6.78 per cent cellulose, 17.48 per cent lignin and 15.98 per cent silica in sundried *Azolla pinnata*. However, Taklimi *et al.,* (1993) observed a much lower level of acid insoluble ash (6.81 per cent), acid detergent fibre (25.24 per cent) and neutral detergent fibre (40.36 per cent) on dry matter basis in *Azolla microphylla.*

2.5.6.1.2 Amino acid profile

The literature on amino acid composition of *Azolla* is scanty and the available literature is summarized and presented in Table 2.

Sanginga *et al.,* (1989) reported the possible variation in amino acid composition by analysing seven *Azolla* species at four different growth phases. All *Azolla* strains contained similar proportions of essential (55 per cent) and non-essential (45 per cent) amino acids. Leucine, lysine, arginine and phenylalanine were the predominant essential amino acids in the various samples whereas the sulphur containing amino acids (methionine and cystine) were present in smaller amounts. They found that *Azolla microphylla* strain was the best source of amino acids on the basis of chemical score index.

Table 2 **Amino acid content of *Azolla pinnata* and *Azolla microphylla***

Amino acid	*Azolla pinnata* [1] μ moles/g FW	*Azolla pinnata* [2] g/100 g DM	*Azolla microphylla* [3] % of protein
Aspartic acid	18.79	-	-
Glutamic acid	49.34	-	-
Asparagine	24.75	-	9.02
Serine	16.38	-	5.62
Glutamine	283.79	-	8.54
Arginine	37.99	0.8	9.24
Valine	33.10	0.8	4.76
Alanine	22.59	-	6.44
Phenylalanine	12.77	-	1.97
Isoleucine	9.13	-	7.76
Ornithine	40.40	-	-
Cystine	-	0.2	0.53
Glycine	-	0.9	3.90
Histidine	-	0.3	3.98
Leucine	-	1.3	8.01
Lysine	-	0.6	7.92
Methionine	-	0.3	1.14
Proline	-	-	1.13
Threonine	-	0.7	4.39
Tyrosine	-	0.5	4.02

. Kannaiyan (1985)
. Ali and Leeson (1994)
. Taklimi *et al.*, (1993)

The total ash and insoluble ash content in several species of *Azolla* samples were found to be variable. The total ash content varied from 10 to 35 per cent. The high insoluble ash content could be because of contamination of sand and silica.

2.5.6.1.3 Mineral Composition

The available information on mineral content of *Azolla* is summarised and given in Table 3.

The calcium content was generally high. Alcantera and Querubin, (1985) recorded 1.05 per cent calcium and 0.43 per cent phosphorus. Similar values were reported by Tamang *et al.,* (1992). Taklimi *et al.,* (1993) recorded a value of 1.05 per cent phosphorus. Buckingham *et al.,* (1978) reported that the level of minerals present in *Azolla filiculoides* was comparable to that of other *Azolla* species.

2.5.6.2 Pigments

Williams (1992) reported that the ß-carotene in plants was a poor source of pigment for poultry but excellent one for milk fat, whereas the xanthophyll was efficiently utilized and deposited in egg yolk and poultry fat instead of ß-carotene.

Limcango (1989) showed that water plants contain 500-600 mg of xanthophylls per kg of feed. If processed properly, leaf meals are good pigmenting agents because of the presence of several xanthophylls.

Table 3 **Mineral content *Azolla* as reported in literature (% DMB)**

Minerals	Subudhi & Singh (1978)	Singh (1978)	Querubin *et al.*, (1986)	Tamang *et al.*, (1992)	Taklimi *et al.*, (1993)
Calcium	1.8	0.4 - 1.0	1.67 - 2.07	1.54	1.70
Phosphorus	1.0	0.5 - 0.9	0.46 - 0.77	0.35	1.05
Potassium	-	2.0 - 4.5			
Magnesium	-	0.5 - 0.6			
Manganese	-	0.11 - 0.16			
Iron	-	0.06 - 0.26			

The chlorophyll content of *Azolla pinnata* was found to be 0.34 to 0.55 per cent on dry matter basis and recorded more yellowish egg yolk due to *Azolla* feeding as compared to birds fed commercial feed (Singh and Subudhi, 1978).

Querubin *et al.*, (1986) showed that the dehydroxy pigment of xanthophyll in protein *Azolla* meal was responsible in pigmenting the shanks of the boilers at increasing levels of *Azolla* in the diet (0, 5, 10 and 15.1 per cent).

Layers fed diets with *Azolla* meal had significantly higher yolk colour score than the control. Increasing the level of *Azolla* meal (0, 5, 10 and 15 per cent) in the diet significantly increased the yolk colour score of eggs (Bastian, 1987).

2.5.6.3 Feeding value of *Azolla*

The literature on the use of *Azolla* as a feed for cattle, pigs and poultry is limited. Moore (1969) reported the use of *Azolla* as a feed for pigs and ducks. Singh and Subudhi (1978) reported similar use of *Azolla* in Indonesia and Vietnam, while Liu Chang Chu (1979) had reported the use of fresh, cooked or fermented *Azolla* for feeding pigs in China.

A review of literature on the utilization of *Azolla* as a feed for livestock and poultry reveals that *Azolla* has not been fully investigated for its feeding value and hence its usefulness as a feedstuff is not clearly established.

2.5.6.3.1 Cattle

Singh *et al.,* (1983) conducted growth cum digestibility trial on twenty crossbred heifers using *Azolla*. *Azolla* was incorporated in the concentrate mixture to replace 0, 30, 60 and 100 per cent dry matter. The daily gain in body weight of heifers in the four diets was 138, 200, 273 and 333g respectively. The daily feed cost was Rs.1.90, 1.84, 1.41 and 1.38 respectively. Unlike fresh *Azolla*, the sun dried *Azolla* was found to be poorly palatable when offered as a sole ingredient.

In another experiment Nik-khan and Motaghi (1992) fed concentrates containing 0, 15, 25 and 35 per cent *Azolla* to 16 lactating cows. The rations were isocaloric and isonitrogenous and there was no significant difference ($P<0.05$) in milk yield and milk fat percentage between the treatment groups. The levels of protein, ash, lactose and total solids in milk were also not affected. Further, this group of workers failed to note any palatability related problems while feeding *Azolla*.

2.5.6.3.2 Goats

Parnerkar *et al.,* (1986) conducted a metabolism trial on six Patanwadi ewes of similar body weight in order to determine the nutritive value of sun dried *Azolla pinnata*. The dry matter intake was 67.60 ± 3.64 g / kg $W^{0.75}$. The balances of N and P were positive and that of Ca was marginally negative. The DCP and TDN values were estimated as 10.20 per cent and 45.41 per cent respectively.

Tamang *et al.,* (1992) assessed the feeding value of *Azolla* in goats in a metabolism trial. Sun dried *Azolla* was offered as a sole feed for 17 days to the experimental animals. The dry matter consumption was found to be low (2.32 kg/

100 kg live weight). The digestibility of crude protein and crude fibre was 56.60 and 41.53 per cent respectively. The animals maintained positive N and P balances but Ca balance was marginally negative. The DCP and TDN values of *Azolla* were 8.70 and 51.78 per cent respectively. Although *Azolla* was rich in nutritive value these authors found it unpalatable to goats.

Tamang and Samanta (1993) replaced 0, 10, 20 and 50 per cent of a standard goat diet with sundried *Azolla* in a 90 day growth trial. At 50 per cent replacement the goats suffered profuse diarrhoea. The remaining groups did not show any significant difference in DM intake and weight gain. The Ca and P balances were similar on all three diets. The diets containing various levels of *Azolla* did not produce any significant effect on carcass characteristics, haematological and biochemical parameters. The production of VFA in the rumen was highest with 20 per cent *Azolla* ($P < 0.01$). They suggested that upto 20 per cent *Azolla* can be incorporated in concentrate feed for goats without any deleterious effect.

2.5.6.3.3 Swine

Alcantera and Querubin (1989) raised weaned piglets on diets containing 0, 5, 10 and 15 per cent *Azolla* meal replacing rice bran. The feed conversion efficiency on various diets was significantly different and the average daily gain of piglets fed 10 per cent *Azolla* diet was 10 to 14 per cent higher as compared to the gain in other groups. In another trial, grower diets containing 0, 10, 20 and 30 per cent *Azolla* replacing rice bran was fed to pigs. There were no significant differences in growth, but feed conversion efficiency decreased in 30 per cent *Azolla* diet but not in 20 per cent diet which was similar to that of the control.

Pigs with a live weight of 20-90 kg were fed with sugarcane juice based diets supplemented with soyabean oil meal based concentrate containing 15 and 30 per cent of protein as *Azolla* protein, and growth rate and feed efficiency were reported similar in all groups by Becerra *et al.*, (1990).

2.5.6.3.4 Rabbits

Gualtieri *et al.*, (1988) conducted a trial on 35 four weeks old rabbits using isonitrogenous diets equal in fibre containing soyabean oil meal (SBM). The experimental rations contained *Azolla* replacing SBM at 0, 50 and 100 per cent. The replacement of all SBM with *Azolla filiculoides* meal significantly decreased daily weight gain, feed conversion efficiency and dressing percentage. However carcass quality was not affected.

Wittouck *et al.*, (1992) fed 30 days old rabbits to appetite on a basal diet of 15 per cent oatmeal, 40 per cent dried lucerne, 10 per cent soyabean meal, 25 per cent sunflower meal, 8 per cent molasses and 2 per cent minerals alongwith vitamins. Dried *Azolla pinnata* was added at 20, 40 or 60 per cent levels replacing 10, 20 or 40 per cent dried lucerne. The diets with 40 and 60 per cent dried *Azolla pinnata* depressed weight gain and the depression being greater at 60 per cent level. It was concluded that *Azolla* could be added upto 20 per cent in a balanced diet for growing rabbits.

Sreemannarayana *et al.,* (1993) fed young Newzealand and Russian Grey giant rabbits a commercial pelleted feed mixture containing *Azolla* at 0, 10, 15 or 20 per cent. The average daily weight gain was 25.3, 26.7, 25.3 and 27.3 (g) respectively and the mean daily DM intake was 72.3, 79.3, 68.6 and 83.2 (g) suggesting that *Azolla* can be included in the diet for growing rabbits upto 20 per cent without any adverse effect.

2.5.6.3.5 Ducks

The effects of feeding fresh *Azolla* as a partial replacement to traditional palay - snail - shrimp based rations for laying mallard ducks was studied by Escobin and Alejar (1987). There was no significant difference in performance among all treatments containing 0, 20, 30 and 40 per cent fresh *Azolla*. Likewise egg quality was generally not affected by the inclusion of *Azolla* in the mallard duck diet.

2.5.6.3.6 Fishes

Pullin *et al.,* (1988) incorporated ground sundried *Azolla pinnata* meal in the experimental diets of fishes at different levels (8.50, 17.00, 25.46, 34.00 and 42.45 per cent) in an attempt to replace fish meal in the diet. The rations were fed to Nile Tilapia fry (mean body weight 14.9 mg in Experiment 1 and 11.2 mg in experiment 2) at 45 per cent of fish biomass daily for seven weeks. The results of the two experiments showed that *Azolla* meal is a suitable component of diets for Nile tilapia fry. The growth and feed conversion ratios improved as the level of dietary *Azolla* meal increased. The survival rates were not influenced by the levels of *Azolla* in the diets.

2.5.7 Poultry

2.5.7.1 Broilers

Querubin *et al.,* (1986) conducted a feeding trial using 96 day-old broiler chicks to establish the optimum level of *Azolla* meal in broiler ration. The birds were randomly allotted to four dietary treatments with 0, 5, 10 and 15 per cent *Azolla microphylla* meal. The average body weight gain of the birds was not significantly affected due to the presence of *Azolla* meal in the diet, however, the feed consumption of the birds increased significantly (P < 0.05) at 5 per cent level and consistently increased with higher level of *Azolla* meal but with a reduction in feed efficiency. The coefficient of digestibility of DM, CP, NFE and dressing percentage did not differ significantly among the treatments. The Roche colour value showed that the dehydroxy pigment of xanthophyll in *Azolla* meal was effective in pigmenting the shanks of birds at increasing levels in the diet. They concluded that inclusion of *Azolla* meal in broiler ration was the best at 10 per cent although the growth rate of birds was not affected upto 15 per cent level.

Taklimi *et al.,* (1993) carried out a feeding trial of 6 weeks duration in broilers using *Azolla* meal. The diet contained 0, 10 and 15 per cent *Azolla* meal with or without cellulase enzyme replacing corn and peanut cake mixture on weight by weight basis. The body weight of birds fed *Azolla* leaf meal at 5 and 10 per cent levels was significantly better (P < 0.05) than control and the group containing 15 per cent *Azolla* leaf meal irrespective of enzyme supplementation. The feed efficiency did not differ significantly among various treatment groups. The dressing percentage remained unaffected with either level of inclusion of *Azolla* leaf meal or enzyme supplementation.

Sundararasu *et al.*, (1994) compared the use of dried *Azolla*, Sesbania and Leucaena meals in broiler diets (starter and finisher) at 6 percent level and the rations were made isonitrogenous. The performance of broilers fed these diets were compared with a group fed standard broiler ration. The body weight at 8th week on *Azolla* ration was 1340 (g) as compared to 1318 in Leucaena while those fed Sesbania weighed significantly lesser (1118.5). The feed efficiency and livability of the group fed *Azolla* meal was comparatively better than the other groups. The carcass traits did not differ significantly between groups.

2.5.7.2 Layers

Subudhi and Singh (1978) fed fresh *Azolla* to 6 weeks old White Leghorn birds at levels of 16, 12.5 and 5 per cent along with 50, 75 and 100 per cent feed respectively on dry weight basis. The birds liked green *Azolla* more than red ones and plants with less roots were preferred. At the end of 14 weeks of age the increase in weight of birds receiving 16 per cent *Azolla* was lower than the control whereas the growth in the 12.5 and 5 per cent *Azolla* was better than the control. The mean body weight gain of birds at the end of the trial was 494 g, 344 g, 518 g and 614 g for 0, 16, 12.5 and 5 per cent *Azolla* diets respectively. There was no indication of toxicity in any of the treatment groups. All the birds were in positive nitrogen balance. The egg production in the treatment of 75 per cent feed plus 12.5 per cent *Azolla* was identical to the control.

Bastian (1987) compared rations containing three levels of *Azolla* (5, 10 and 15 per cent) of two different species namely *Azolla microphylla* and *Azolla pinnata* in the layer rations and fed for a period of 12 weeks. The metabolizable energy of *Azolla microphylla* was found to be higher than *Azolla pinnata*. The digestibility of CP, CF, EE and NFE were not significantly influenced by *Azolla* species. Increasing the level of *Azolla* meal in the diet did not significantly affect digestibilities of CP, CF and NFE, whereas the metabolizable energy of diets decreased consistently as the level of the meal increased. The egg production, egg size and feed consumption of the layers as well as the shell thickness of eggs were not increased significantly. The yolk colour score of eggs increased but had no effect on feed efficiency. The inclusion of 15 per cent *Azolla* meal reduced significantly the body weight of layers. The protein digestibility increased with *Azolla microphylla* but decreased with *Azolla pinnata* as the level of the meal was increased in the diet. There was no significant interaction between species and the level of *Azolla* meal with respect to the parameters studied.

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

Azolla pinnata was collected from four locations in Tamilnadu, namely; Tamilnadu Rice Research Institute, Aduthurai, Blue green algae project, Aduthurai, Tamilnadu Agricultural University, Coimbatore and Agricultural College, Madurai, and processed for proximate analysis, fibre fractions and mineral content. Six samples were collected from each location, sundried, processed independently and analysed in duplicate. *Azolla* required for conducting the feeding experiments was cultivated in cement tanks at Poultry Research Station, Nandanam, Madras (Plate 1, 1a and 1b).

3.1 Collection of samples

All the samples used for proximate analysis were collected on the fifth day of growth. Approximately 500 g sample was collected on each occasion and washed four or five times thoroughly in water to remove the mud and sand sticking on to the leaves and roots. The fresh samples were dried immediately after collection in a hot air oven at 80°C for 24 hours. The dried samples were ground in a Willey mill and stored in air tight containers for further analysis.

Azolla used for the feeding experiments was grown in four concrete tanks of 20 square metre size with soil of eight inches depth and maintaining a 8" to 10" deep water level over the soil. The water was fertilized with 50g of super phosphate and 50 g of urea per week. Four kilos of seed material received from Tamilnadu Rice Research Institute, Aduthurai was used as innoculum. Fresh *Azolla* was harvested

Plate 1 - Cultivation of *Azolla pinnata* - Cement tanks showing *Azolla* growth

Plate 1a - Fresh samples of *Azolla pinnata* soon after collection

Plate 1b - Samples of sundried *Azolla pinnata* used in the feeding trials at weekly intervals over an eight week period, sundried on cement floor and stored in polythene bags for conducting the feeding experiments.

3.2 Chemical analysis

The sundried samples of *Azolla* were analysed for dry matter, crude protein, ether extract, crude fibre, total ash as per the method of AOAC (1984) using Kjeltec (model number 1002), Soxtec (model number 1043) and fibretec (model number 1020) equipments of Tecator, Sweden respectively.

3.2.1 Fibre fractions

The *Azolla* samples were analysed for fibre fractions : acid detergent fibre, neutral detergent fibre, hemicellulose, cellulose and lignin as per the method described by Goering and VanSoest (1970).

3.2.2 Mineral composition

The concentration of four major minerals namely calcium, magnesium, sodium and potassium and trace minerals iron, copper, zinc, manganese and cobalt were estimated in an atomic absorption spectrophotometer as per the procedure prescribed in the reference manual (Perkin Elmer Model 2380 - 1971). The phosphorus content was estimated colorimetrically (Cavell 1955).

Apart from these minerals the concentration of lead in *Azolla* was also estimated in an atomic absorption spectrophotometer. The wave length and other specifications adopted for various minerals in the atomic absorption spectrophotometer are given in the Table 4.

Table 4 **Atomic absorption spectrophotometer specifications adopted for estimation of minerals**

Elements	Wave length nm	Slit width	Flame	Working Standard µg / ml	Integration in seconds
Calcium	423	0.7	Air-acetylene (lean blue)	5.0	1.5
Magnesium	285	0.7	"	0.5	1.5
Sodium	589	0.7	"	1.0	1.0
Potassium	766	2.0	"	2.0	1.5
Iron	248	0.2	"	5.0	1.5
Copper	325	0.7	"	5.0	1.0331
Zinc	214	0.7	"	0.5	1.5
Manganese	279	0.2	"	3.0	1.0
Cobalt	241	0.2	"	5.0	1.0
Lead	283	0.7	"	20.0	1.0

3.2.3 Energy estimation

The energy values of various samples of *Azolla* for gross energy estimation was done in an adiabatic bomb calorimeter. The samples of *Azolla* and droppings collected in the metabolizable energy trial using layers were also analysed in the bomb calorimeter for energy values.

3.2.4 Amino acid assay

The amino acid assay of *Azolla pinnata* was estimated at National facility for marine cynobacteria, Bharathidasan University, Trichirapalli. One sample of *Azolla* in duplicate was analysed using HPLC auto analyser as per the procedure of Rajaram and Janardhanan (1990). The finely ground *Azolla pinnata* sample was defattened using petroleum ether in ether extraction apparatus for 8 - 10 hours. The defattened sample was dried and 10 per cent trichloroacetic acid added to precipitate the protein. The trichloroacetic acid in the sample was removed by addition of ether. The sample was then dried and the protein in the sample was hydrolysed in 6N hydrochloric acid. A measured quantity of hydrolysate was injected into the column of HPLC. The peak values of individual amino acids were recorded and compared with standards to quantify the amino acids.

3.3 Biological Experiments

3.3.1 Determination of metabolizable energy in layers. (Experiment - 1)

The apparent and true metabolizable energy values of samples of *Azolla* grown at Poultry Research Station, Nandanam, were estimated with twelve single comb White Leghorn roosters of six months of age as per the method of Sibbald (1976). The ground *Azolla* meal was made into pellets of 10 mm size each weighing approximately 1.5 to 2 grams. The twelve roosters of similar body weight were randomly allotted to two treatments with six birds in each group. The birds were housed in individual cages and allowed an adaptation period of seven days. Feed and water were provided *ad libitum* during the adaptation period.

The birds were starved for 24 hours prior to the starting of the experiment. Six birds in a group were individually weighed and force fed approximately 25 grams of *Azolla* pellets with minimum strain to the birds. The six birds in the other group were without any feed and used as control. The droppings was collected after 24 hour of feeding on a plastic sheet tied on to each cage. An *ad libitum* supply of water was made available during the 24 hour experimental period. The excreta was collected quantitatively without any external contamination like feather and feed particles. The collected excreta was weighed individually and made into a homogenous mass and samples taken for moisture estimation. The dried samples were ground using 20 mm seive and stored for further analysis. The energy and nitrogen content of the feed and excreta were determined and metabolizable energy values calculated as per the formula given below :

$$\text{ME (kcal/g)} = \frac{GE_f \times X - Yef}{X}$$

$$\text{TME (kcal/g)} = \frac{(GEf \times X) - (Yef - Yec)}{X}$$

where

GE_f	is the gross energy of the feedstuff (kcal/g)
X	is the weight of feedstuff (g)
Ye_f	is the energy voided as excreta by the fed birds
Ye_c	is the energy voided as excreta by the unfed birds

3.3.2 Feeding value of *Azolla* in chick mash from 0-8 weeks of age. (Experiment-2)

Ninety six day-old female chicks of a pure line synthetic egg strain belonging to a single hatch were weighed individually, wing banded and randomly distributed to eight groups of 12 chicks each. They were subjected to four dietary treatments with two replications for each treatment. Rations were formulated using ICAR (1985) standards. Sundried *Azolla* meal was included at 0, 5, 10 and 15 per cent levels in four rations. *Azolla* replaced a protein mix containing groundnut cake and wheat bran in the proportion of 50:50. The ingredient composition of chick mash used in this experiment is given in Table 5.

Feed and water were made available at all times. The birds were reared in deep litter pens using electrical bulbs as heat source. The chicks were vaccinated against Marek's and New Castle disease on the day of hatch and on sixth day respectively. A record of weekly weight gain, feed intake, feed efficiency and mortality were maintained throughout the eight week experimental period.

3.3.3 Feeding value of *Azolla* in chick mash for commercial layers from 0-8 weeks of age. (Experiment - 3)

Ninety six day-old female chicks of a commercial strain *(bovans)* belonging to a single hatch obtained from Pioneer hatcheries were used in this study. The experimental design, the level of incorporation of *Azolla* in the experimental rations, the management and parameters studied remained the same as in experiment 2. This study was primarily conducted to confirm the findings of the earlier experiment and to study the influence of strain, if any, on the feeding value of *Azolla*.

Table 5 **Per cent ingredient composition of brooder rations - Experiment 2 & 3**

Ingredients	% *Azolla* in the ration			
	0	5	10	15
Maize	47	47	47	47
Cumbu (Bajra)	9	9	9	9
Sunflower oil cake	5	5	5	5
Fish meal	8	8	8	8
Ground nut oil cake	14	14	14	14
Protein mix[1]	15	10	5	-
Dried *Azolla*	-	5	10	15
Mineral mixture[2]	2	2	2	2
Vitamin mixture[3] (g)	20	20	20	20
Coccidiostat[4] (g)	50	50	50	50
Calculated values				
Crude protein %	20.08	20.06	20.04	20.01
ME kcal / kg	2698	2678	2658	2638

1. A mixture of solvent extracted ground nut oil cake and wheat bran in the proportion of 50:50 to have 28.2% crude protein and ME 1700 kcal/kg.

2. Supplied per kg of diet : Calcium 6.4 g; Phosphorus 1.2 g; Manganese 54 mg; Iodine 2 mg; Zinc 52 mg; Flourine 6 mg; Copper 2 mg and Iron 20 mg.

3. Supplied per kg of diet : Vitamin A 8250 IU; Vitamin D3 1200 IU; Vitamin B2 5 mg and vitamin B6 6 mg.

4. Contained sulphaquinoxaline 18.7% W/W and Diavendine 3.3% W/W

3.3.4 The value of *Azolla* in grower mash from 9-20 weeks of age. (Experiment - 4)

One hundred and eighty four White Leghorn Meyer strain birds of 8 weeks of age and beloriging to a single hatch were weighed individually and randomly distributed to eight groups of 24 chicks each. The eight groups of birds were subjected to four dietary treatments with two replicates for each treatment. Sun dried *Azolla* meal was incorporated in the four rations at 0, 5, 10 and 15 per cent levels. *Azolla* was used to replace a protein mix containing groundnut cake and wheat bran in the ratio of 50:50. The birds were reared in deep litter pens using paddy husk as litter material. The ingredient composition of the experimental rations is furnished in Table 6.

The birds were provided with *ad libitum* supply of feed and water. The birds were vaccinated with New Castle disease virus 'K' strain on the eight week prior to start of the experiment. A record of biweekly weight gain, feed intake, feed efficiency, age at maturity, weight at maturity, weight of first egg and livability were maintained.

3.3.5 Feeding value of *Azolla* in layer mash from 21-40 weeks of age. (Experiment-5)

Two hundred White Leghorn layers of Meyer strain aged 20 weeks were weighed individually and distributed at random into eight groups of twenty five birds each. They were subjected to four dietary treatments with two replicates for each trial. The four experimental rations were prepared containing sundried *Azolla* meal at 0, 5, 10 and 15 per cent levels replacing a protein mix of groundnut cake and rice polish in the proportion of 50:50. The ingredient composition of the experimental rations is furnished in Table 7.

Table 6 Per cent ingredient composition of grower rations - Experiment 4

Ingredients	% *Azolla* in the ration			
	0	5	10	15
Maize	50	50	50	50
Cumbu (Bajra)	13	13	13	13
Groundnut Oil Cake	3	3	3	3
Wheat Bran	9	9	9	9
Fish Meal	8	8	8	8
Protein mix[1]	15	10	5	-
Dried *Azolla*	-	5	10	15
Mineral mixture[2]	2	2	2	2
Vitamin mixture[3] (g)	20	20	20	20
Coccidiostat[4] (g)	50	50	50	50
Calculated Values				
Crude protein %	16.29	16.27	16.25	16.23
ME kcal / kg	2647	2627	2607	2587

1. A mixture of ground nut oil cake and wheat bran in the proportion of 50:50
2. Supplied per kg of diet :
   Calcium 6.4 g; Phosphorus 1.2 g; Manganese 54 mg; Iodine 2 mg; Zinc 52 mg Flourine 6 mg; Copper 2 mg and Iron 20 mg
3. Supplied per kg of diet : vitamin A 8250 IU; vitamin D3 1200 IU; vitamin B2 5 mg; and vitamin B6 6 mg
4. Coccidiostat contained sulphaquinoxaline 18.7 W/W and Diavendine 3.3% W/w

Table 7 Per cent ingredient composition of Layer Rations - Experiment 5

Ingredients	% *Azolla* in the ration			
	0	5	10	15
Maize	50	50	50	50
Tapioca flour	7	7	7	7
Ground nut oil cake	12	12	12	12
Fish meal	8	8	8	8
Vegetable Oil	1	1	1	1
Dried *Azolla*	0	5	10	15
Protein mix[1]	15	10	5	-
Shell grit	5	5	5	5
Mineral mixture	2	2	2	2
Vitamin mixture[3] (g)	20	20	20	20
Choline chloride[4] (g)	100	100	100	100
Calculated values				
Crude protein %	17.33	17.34331	17.36	17.36
ME kcal / kg	2819	2754	2684	2624

1. A mixture of ground nut oil cake and rice polish in the proportion of 50:50
2. Supplied per kg of diet :
   Calcium 6.4 g; Phosphorus 1.2 g; Manganese 54 mg; Iodine 2 mg; Zinc 52 mg Flourine 6 mg; Copper 2 mg and Iron 20 mg
3. Supplied per kg of diet : vitamin A 8250 IU; vitamin D3 1200 IU; 331 vitamin B2 5 mg; and vitamin B6 6 mg

The birds were reared in deep litter houses using paddy husk as litter material. The feeding, watering and the managemental conditions were same as in the previous experiment. A record of biweekly weight gain, feed intake and feed efficiency was maintained throughout the experimental period of 21-40 weeks of age. The performance of layers for the five 28 day periods was recorded. Six eggs at random in each group on the last 3 days of each 28 day periods were taken for egg quality studies. In addition to egg production, egg weight, feed intake and feed efficiency, the following egg quality parameters were studied.

a) Shape index
b) Albumen index
c) Haugh unit
d) Yolk index
e) Yolk colour
f) Shell thickness

3.3.5.1 Shape Index

The length and width of eggs were measured upto one tenth of a mm with Vernier callipers. The length was measured as the distance between the poles, and the width as the distance at maximum circumference of egg. Shape index was calculated by the formula given below :

$$\text{Shape index} = \frac{\text{Greatest width x 100}}{\text{Greatest length}}$$

3.3.5.2 Albumen Index

The eggs were broken out on a glass plate. The albumen height was measured with Ames tripod micrometer as described by Haugh (1937). The long and short axis of the apparent thick white were measured with Vernier callipers and mean width calculated. The albumen index was calculated using the formula prescribed by Heimen and Carver (1936).

$$\text{Albumen Index} = \frac{\text{Albumen Height}}{\text{Average Diameter}} \times W$$

Where

H is the height (mm)

3.3.5.3 Haugh unit

Haugh unit was calculated taking into account the weight and height of egg using USDA egg quality sliding rate.

3.3.5.4 Yolk index

The mean width and maximum height of yolk were measured by Vernier callipers and Ames tripod micrometer respectively and yolk index was calculated as follows :

$$\text{Yolk index} = \frac{\text{Yolk width (mm)}}{\text{Yolk height (mm)}}$$

3.3.5.5 Yolk colour

Yolk colour matchings were done by using Roche yolk colour fan

3.3.5.6 Shell thickness

Shell thickness measured as per the method suggested by Muller and Scott (1940) using micrometer screw gauge with anvil jaws. Measurements of egg shell thickness were taken at three points, namely equatorial region, narrow and broad ends and the mean calculated.

3.3.6 Feeding higher levels of *Azolla* in layer rations from 21-40 weeks of age. (Experiment - 6)

The data collected in trial 5 suggest that *Azolla* could be used in layer rations at 15 per cent level without any adverse effect. Encouraged by this observation another experiment was conducted to study the effect of higher levels of *Azolla* on layer performance.

One hundred and forty four White Leghorns Meyer strain layers of 20 weeks of age were weighed individually and distributed at random to eight groups of 18 birds each and subjected to four dietary treatments with two replicates for each. The rations were pelleted in a pellet mill at the Poultry Research Station, since the mixed rations were bulkier. The pelleted feed was used throughout the experimental period. Sundried *Azolla* meal was incorporated at 0, 15, 20 and 25 per cent levels replacing a protein mix containing groundnut cake and wheat bran in the ratio of 50:50. The ingredient composition of experimental rations is furnished in Table 8.

Table 8 **Per cent ingredient composition of Layer mashes Experiment - 6**

Ingredients	% *Azolla* in the ration			
	0	15	20	25
Maize	56	56	56	56
Ground nut oil cake	4	4	4	4
Fish meal	8	8	8	8
Protein mix[1]	25	10	5	-
Dried *Azolla*	-	15	20	25
Shell grit	5	5	5	5
Mineral mixture[2]	2	2	2	2
Vitamin mixture[3] (g)	20	20	20	20
Choline chloride 50 %[4] (g)	100	100	100	100
Calculated values				
Crude protein %	17.26	17.19	17.16	17.14
ME kcal / kg	2617	2557	2537	2517

1. A mixture of ground nut oil cake and wheat bran in the proportion of 50:50
2. Supplied per kg of diet : Calcium 6.4 g; Phosphorus 1.2 g; Manganese 54 mg; Iodine 2 mg; Zinc 52 mg; Flourine 6 mg; Copper 2 mg and Iron 20 mg
3. Supplied per kg of diet : vitamin A 8250 IU; vitamin D3 1200 IU; vitamin B2 5 mg; and vitamin B6 6 mg

The birds were housed in a two tier Californian type cages with three birds in each cage. An *ad libitum* supply of feed and water was made available throughout the experimental period. A record of weight gain, feed intake and feed efficiency were maintained for five 28 day periods. In addition, the data on egg production and egg quality were collected using similar procedure adopted in experiment 5. At the end of 40th week, four birds from 0 and 15 per cent *Azolla* groups were sacrificed for examination of internal organs. Pieces of liver, lung, spleen and kidney were processed in 10 per cent formalin for histopathalogical examination.

3.3.7 Nitrogen balance trial

At the end of 40th week of age twenty four birds were selected at random at the rate of six birds per treatment and maintained in separate cages for balance studies. The birds were fed individually with the same experimental pellets containing 0, 15, 20 and 25 per cent sundried *Azolla*. A three day collection period was adopted. A record of feed intake and excreta voided for 24 hours period was maintained for each bird individually for three consecutive days. Enamel trays wrapped with a polythene sheet and containing a small quantity dilute hydrochloric acid was kept below each cage for collection of excreta. The quantity of feed consumed and excreta voided for 24 hours period was separately maintained. Representative samples of fresh excreta was taken after uniform mixing for estimation of nitrogen and moisture content. The three day samples were pooled for six birds individually and six pooled samples were analysed to calculate nitrogen balance.

3.3.8 Economics

The cost of egg production in terms of return over feed cost was calculated by taking into account the number of eggs laid and total cost of feed consumed. The expenditure in terms of labour, electricity and other overhead charges were treated as same for all treatment groups and hence not taken into consideration. The cost of *Azolla* is taken as Rs. 0.50 / kg for the purpose of this calculation.

3.3.9 Statistics

The data collected from various experiments and chemical analysis were subjected to analysis of variance for significance under completely randomized design as per the method of Snedecor and Cochran (1967).

Results and Discussion

CHAPTER 4

RESULTS AND DISCUSSION

The results of various chemical analysis conducted in connection with the nutritional evaluation of *Azolla* in White Leghorn birds are presented and critically discussed in this chapter.

4.1 Chemical analysis

4.1.1 Drymatter content of fresh *Azolla*

The per cent dry matter content of fresh *Azolla* was 11.88 ± 1.22, 11.84 ± 1.14, 13.55 ± 1.22 and 11.31 ± 1.28 for the samples collected from TRRI, BGAP and TNAU Coimbatore and Madurai respectively. The overall drymatter for all four samples was 12.16 ± 1.21. Similar values on drymatter content were reported by Islam (1993), but Moore (1969) and Singh (1978) have reported much lower values in the range of 4.8 to 7.7 per cent.

4.1.2 Proximate composition of sundried *Azolla*

The proximate composition of 12 samples of *Azolla pinnata* collected from each of the four different sources is given in Table 9.

The crude protein in all four samples ranged between 21.99 ± 0.57 to 27.50 ± 0.54 with an overall mean of 25.46 ± 0.69. The ether extract fraction varied from 1.61 ± 0.15 to 2.09 ± 0.11 with an overall mean of 1.88 ± 0.11. The per cent crude

Table 9 The proximate composition of sundried *Azolla pinnata* collected from different locations in Tamilnadu (per cent dry matter basis, mean* ± SE)

Composition	TRRI	BGAP	TNAU		Overall Mean ± SE
			Madurai	Coimbatore	
Dry matter	90.59 ± 0.16	89.52 ± 0.82	88.23 ± 0.89	91.69 ± 0.85	90.01 ± 0.74
Crude protein[1]	27.50[b] ± 0.54	21.99[a] ± 0.57	26.62[b] ± 0.76	25.73[b] ± 0.86	25.46[b] ± 0.69
Ether extract	1.83 ± 0.05331	1.61 ± 0.15	2.09 ± 0.11	1.99 ± 0.14	1.88 ± 0.11
Crude fibre[2]	11.81[a] ± 0.48	14.22[b] ± 0.56	10.84[a] ± 0.54	12.97[a] ± 0.62	12.46[a] ± 0.55
Nitrogen free extract[1]	44.81[b] ± 0.55	48.48[c] ± 0.41	46.60[b] ± 0.48	40.67[a] ± 0.28	45.14[b] ± 0.44
Total ash[1]	14.05[a] ± 1.06	13.70[a] ± 1.04	13.85[a] ± 1.02	18.64[c] ± 0.98	15.06[b] ± 1.03
Insoluble ash[1]	5.39[a] ± 0.62	5.50[a] ± 0.64	5.62[a] ± 0.57	10.70[b] ± 0.56	6.80[a] ± 0.60

1 Mean with different superscript in a row differ significantly ($P<0.01$).
2 Mean with different superscript in a row differ significantly ($P<0.05$).

TRRI - Tamilnadu Rice Research Institute, Aduthurai
BGAP - Blue green algae project, Aduthurai
TNAU - Tamilnadu Agricultural University

fibre content ranged from 10.84 ± 0.54 to 14.22 ± 0.56 with an average concentration of 12.46 ± 0.55. The total ash and acid insoluble ash content of the four samples varied between 13.70 ± 1.04 - 18.64 ± 0.98 and between 5.39 ± 0.62 - 10.70 ± 0.56 respectively in that order. The overall mean for per cent total ash and acid insoluble ash were 15.06 ± 1.03 and 6.80 ± 0.60 respectively. The acid insoluble ash content was highest in the samples obtained from TNAU Coimbatore, while the samples from BGAP was high in crude protein and ether extract. The samples from TNAU Madurai had the lowest crude fibre content. The earlier reports by Watanabe *et al.,* (1977), Singh (1979), Taklimi *et al.,* (1993), Querubin (1986) and Buckingham *et al.,* (1978) are also similar to the values reported in this study.

The differences in the proximate components varying among the four samples analysed could be due to the differences in the stage of maturity, age at harvest, due to differences in cultivation methods, method of harvesting, contamination with sand and silica, water mineral content, type and quantity of fertilizer applied, strain or variety differences and differences of agroclimatic conditions. It may be noted that there is no major variation or differences in proximate composition among the four samples analysed.

4.1.3 Fibre fractions

The data on fibre fractions in *Azolla* are given in Table 10. The overall mean of neutral detergent fibre content in *Azolla* was 42.39 ± 0.38 and the range of distribution varied from 40.54 ± 0.48 to 45.54 ± 0.35. The acid detergent fibre values ranged from 26.89 ± 0.17 to 30.33 ± 0.17 with an overall mean of 27.94 ±

Table 10 **The Fibre fractions of sundried *Azolla pinnata* collected from different locations in Tamil Nadu (% DMB, mean* ± SE)**

	TRRI	BGAP	TNAU		Overall Mean ± SE
			Madurai	Coimbatore	
Neutral detergent fibre	$41.12^a \pm 0.25$	$45.54^a \pm 0.35$	$40.54^a \pm 0.48$	42.34^a 0.39	$42.39^a \pm 0.38$
Acid detergent fibre	27.01 ± 0.20	30.33 ± 0.17	26.89 ± 0.17	27.64 ± 0.11	27.94 ± 0.17
Hemicellulose	14.11 ± 0.09	15.21 ± 0.08	13.65 ± 0.10	14.70 ± 0.09	14.45 ± 0.09
Cellulose	12.00 ± 0.14	13.11 ± 0.03	12.35 ± 0.08	12.19 ± 0.07	12.44 ± 0.09
Lignin	6.54 ± 0.08	7.27 ± 0.09	7.01 ± 0.08	6.89 ± 0.03	6.93 ± 0.07

Means with different superscript in a row differ significantly ($P < 0.05$)

TRRI - Tamilnadu Rice Research Institute, Aduthurai
BGAP - Blue green algae project, Aduthurai
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0.17. The mean values of cellulose, hemicellulose and lignin for all four samples were 12.44 ± 0.09, 14.62 ± 0.09 and 6.93 ± 0.07 respectively. The range of distribution for the same three fibre fractions was 12.00 ± 0.14 - 13.11 ± 0.03, 13.65 ± 0.10 - 15.21 ± 0.08 and 6.54 ± 0.08 - 7.27 ± 0.09 respectively in that order. The level of fibre fractions analysed in this study was similar to the reports of Singh *et al.,* (1983), Taklimi *et al.,* (1993) and Tamang *et al.,* (1992). However higher values of some fractions and lower values for some other fractions were reported by Tamang *et al.,* (1992), Buckingham *et al.,* (1978) and Mohanpur (1992). The reasons for the observed variation between the four samples of *Azolla* analysed and the differences as noticed with the published earlier reports could be due to differences in the agroclimatic conditions, stage of growth and maturity and the influence of other possible contaminants as referred earlier.

4.1.4 Mineral Profile

The concentration of calcium, phosphorus, sodium, potassium, magnesium, manganese, copper, iron, zinc and lead content of samples of *Azolla pinnata* collected from four different sources is given in Table 11.

The per centage of calcium ranged from 1.16 ± 0.23 to 1.71 ± 0.20 with a mean value of 1.48 ± 0.18. The phosphorus content ranged from 0.36 ± 0.11 to 0.76 ± 0.09 with an average of 0.64 ± 0.12. The mean magnesium, sodium and potassium content was 0.78 ± 0.11, 0.16 ± 0.05 and 2.56 ± 0.54 respectively. The values reported are similar to the observations made by Querubin *et al.,* (1986), Tamang *et al.,* (1992) and Takilimi *et al.,* (1993). Others like Subudhi and Singh, (1978) and Singh (1989) have reported variable results, either low or high.

Table 11 **Concentration of certain minerals in *Azolla pinnata* collected from different locations in Tamilnadu (% DMB, Mean ± SE)**

Minerals	TRRI	BGAP	TNAU		Overall Mean ± SE
			Madurai	Coimbatore	
Major Minerals (g/100g)					
Calcium	1.50 ± 0.13	1.16 ± 0.23	1.55 ± 0.14	1.71 ± 0.20	1.48 ± 0.18
Phosphorus[1]	0.76^b ± 0.09	0.70^b ± 0.09	0.36^a ± 0.11	0.75^b ± 0.17	0.64^b ± 0.12
Magnesium	0.80 ± 0.09	0.81 ± 0.09	0.74 ± 0.10	0.75 ± 0.14	0.78 ± 0.11
Sodium	0.14 ± 0.06	0.17 ± 0.08	0.15 ± 0.02	0.16 ± 0.09	0.16 ± 0.05
Potassium[2]	2.47^a ± 0.87	3.49^b ± 0.37	2.64^a ± 0.47	1.63^a ± 0.23	2.56^a ± 0.54
Trace Minerals (mg/kg)					
Iron	1325 ± 51.50	1351 ± 20.42	1260 ± 31.51	1370 ± 34.60	1321 ± 39.53
Copper	14.76 ± 0.87	14.60 ± 0.78	15.32 ± 0.99	15.99 ± 0.67	15.17 ± 0.70
Zinc	55.20 ± 3.48	48.44 ± 5.41	45.21 ± 4.63	50.35 ± 3.84	49.80 ± 4.40
Manganese	352 ± 8.11	369 ± 11.44	320 ± 14.38	390 ± 9.72	356.5 ± 11.16
Cobalt	7.12 ± 0.12	6.72 ± 0.13	6.79 ± 0.10	7.15 ± 0.21	6.95 ± 0.12
Toxic Mineral (mg/kg)					
Lead	0.03 ± 0.002	0.02 ± 0.004	0.03 ± 0.002	0.03 ± 0.001	0.03 ± 0.002

1 Mean with different superscript in a row differ significantly ($P<0.01$)
2 Mean with different superscript in a row differ significantly ($P<0.05$)

TRRI - Tamilnadu Rice Research Institute, Aduthurai
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TNAU - Tamilnadu Agricultural University

The calcium : phosphorus ratio in the four samples varied from 4.3:1 to 1.6:1. The wider variation was recorded only in one sample i.e. from TNAU, Madurai.

Among the trace elements, the concentration of iron ranged from 1260 ± 31.51 to 1370 ± 34.6. The overall mean was 1321 ± 39.53. The range of distribution of copper, zinc, manganese and cobalt was 14.60 ± 0.78 to 15.99 ± 0.67, 45.21 ± 4.63 to 55.20 ± 3.48, 320 ± 14.38 to 390 ± 9.72 and 6.72 ± 0.13 to 7.15 ± 0.21 respectively. The variation in the concentrations of both major and trace elements may be attributed to the possible differences in the concentration of these minerals in water and contamination with sand and soil, differences in fertilizer, agroclimatic conditions and strain and maturity of *Azolla*.

The concentration of the toxic element, lead was very low, at either 0.03 or 0.002 in all the four samples analysed.

4.1.5 Amino acid profile

The amino acid content of *Azolla* determined using HPLC auto analyser on one sample collected from Poultry Research Station, Nandanam is given in Table 12.

The *Azolla pinnata* is high (per cent protein) in arginine (6.73), leucine (8.37), valine (5.01) and Isoleucine (5.20) as compared to groundnut cake. *Azolla* could be a good source of branched chain amino acids but possibly low in sulphur containing amino acids, methionine (1.,09). The concentration of lysine was 4.02.

Table 12 Amino acid composition of *Azolla pinnata* grown at Poultry Research Station, Nandanam

Amino acid	Concentration (% protein)
Alanine	5.51
Arginine	6.73
Aspartic acid	8.74
Glutamic acid	7.54
Glycine	6.01
Isoleucine	5.20
Leucine	8.37
Lysine	4.02
Methionine	1.09
Phenylalanine	1.36
Serine	4.70
Valine	5.01
Threonine	4.49
Tyrosine	4.96

4.2 Feeding Trials

4.2.1 Metabolizable energy content of *Azolla*. (Experiment 1)

The details on the apparent and true metabolizable energy values determined using six roosters are given in the Table 12a. The apparent metabolizable value of *Azolla* for roosters is 1350 kcal / kg, while the true metabolizable energy value is 1659 kcal/kg. The metabolizable energy value of *Azolla* is comparable to that of Ipil-Ipil. The energy value is also similar to that of wheat bran. The dried *Azolla* meal might be useful to replace low energy feeds of industrial and mill byproducts.

4.2.2 Inclusion of *Azolla* in chick mash (0 - 8 weeks). (Experiment - 2)

The data collected from experiment 2 on the feeding value of *Azolla* for chicks are given in Tables 13 to 15 and discussed for two periods namely, 0 - 4 weeks and 5 - 8 weeks of age.

The effect of *Azolla* on weight gain, feed efficiency and protein efficiency ratio in Meyer strain of White Leghorn chicks at 4 weeks of age is given in Table 13. The birds on the basal diet gained 182.3 ± 3.12g while on 5 per cent *Azolla* diet there was a marginal reduction in weight gain at 170.3 ± 5.85g. With higher levels of *Azolla,* namely 10 and 15 per cent levels there was a further reduction in weight gain 153.3 and 138.6g respectively.

Table 12a Metabolizable energy content of *Azolla* (kcal / kg DM)

Roosters	Apparent Metabolizable energy	True metabolizable energy
3001	1325	1635
3002	1410	1710
3003	1270	1575
3004	1300	1605
3005	1380	1690
3006	1415	1740
Mean ± SE	1350.0 ± 24.66	1659.2 ± 28.67

Table 13 **Effect of feeding *Azolla* on the weight gain feed efficiency and protein efficiency in Meyer strain White Leghorn chicks from 0-4 weeks (Mean ± SE) - Experiment 2**

	% *Azolla* in the ration			
	0	**5**	**10**	**15**
Initial weight (g)	30.9 ± 0.35	30.9 ± 0.29	30.8 ± 0.40	30.8 ± 0.36
Final weight (g)	213.2 ± 3.57	201.2 ± 6.24	184.1 ± 4.66	169.4 ± 7.12
Weight gain (g)	182.3 ± 3.12	170.3 ± 5.85	153.3 ± 4.05	138.6 ± 5.52
Feed intake (g)	479	485	502	515
Feed efficiency (feed/ gain)	2.63	2.85	3.27	3.72
Protein intake (g)	96.18	97.29	100.60	103.05
Protein efficiency ratio	1.90	1.75	1.52	1.34
Mortality %	--	--	4.16	4.16

However the differences between the treatments were not statistically significant. The feed intake varied slightly increasing progressively from 479 to 515g on 0 to 15 per cent *Azolla* diet. The feed efficiency declined from 2.63 to 3.72 with increasing levels of *Azolla*. The protein intake and protein efficiency ratio also followed a similar trend. The data is suggestive that, addition of *Azolla* even at 5 per ent level may not be advantageous.

The particulars of weight gain, feed efficiency and protein efficiency ratios for the same set of chicks but from 5 - 8 weeks of age are given in Table 14. The performance at eight weeks were exactly of the same order as at four weeks of age. The weight gain, feed efficiency and protein efficiency ratios decreased progressively from 0 - 15 per cent *Azolla* in the diet.

The cumulative performance of chicks from 0 - 8 weeks is given in the Table 15. The cumulative performance is of the same trend for all the parameters studied as either from 0 - 4 weeks or 5 - 8 weeks of age. At 8 weeks of age the birds on basal diet gained 513.5 ± 12.98g. The weight gain on 5, 10 and 15 per cent *Azolla* diets were 494.8, 461.1 and 406.3g respectively. The loss of weight on 10 and 15 per cent *Azolla* diets as compared to 0 and 5 per cent *Azolla* groups was statistically significant ($P<0.05$). The data on weight gain from 0 - 8 weeks was also given in figure 1. The differences observed among the various other parameters were not statistically significant. The data is not in clear favour of use of *Azolla* as a feed ingredient in chick mash. However the small decrease in weight gain at 5 per cent level could be ignored, if the cost of ration works out cheaper and economically beneficial if *Azolla* could be incorporated in the ration. Further field trials with large numbers of birds have to be conducted to confirm the observations prior to making any specific recommendation. The data clearly indicates that usefulness of *Azolla* as a new feed ingredient in chick mash may be of doubtful value. However, at 5 per cent level the weight difference was not much and if *Azolla* is cheaper it may be used for economic return.

Effect of feeding *Azolla* on weight gain
in WLH Chicks from 0 - 8 weeks
Figure 1

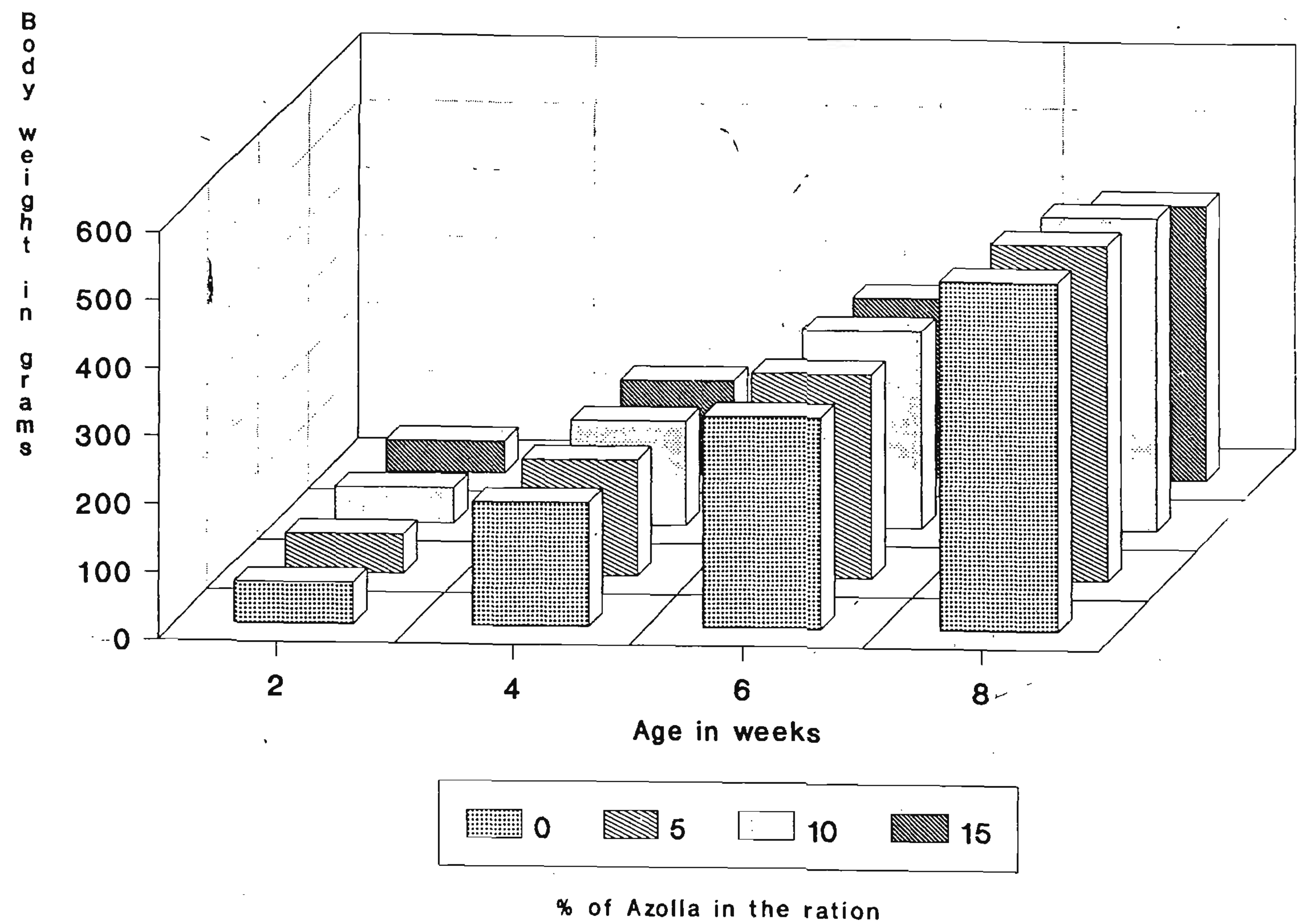


Table 15 Effect of feeding *Azolla* on the weight gain feed efficiency and protein efficiency in Meyer strain White Leghorn chicks from 0-8 weeks (Mean ± SE) - Experiment 2

	% *Azolla* in the ration			
	0	5	10	15
Initial weight (g)	30.9 ± 0.35	30.9 ± 0.29	30.8 ± 0.40	30.8 ± 0.36
Final weight (g)	544.4^{c} ± 13.16	525.7^{c} ± 14.21	491.9^{b} ± 16.17	437.1^{a} ± 15.01
Weight gain (g)	513.5^{c} ± 12.98	494.8^{c} ± 14.12	461.1^{b} ± 15.95	406.3^{a} ± 15.95
Feed intake (kg)	1.85	1.88	1.94	1.95
Feed efficiency (feed/ gain)	3.60	3.80	4.21	4.81
Protein intake (g)	371.18	376.09	381.10	383.15
Protein efficiency ratio	1.38	1.31	1.20	1.06
Mortality %	-	-	4.16	4.16

Mean values with different superscript in a row differ significantly ($P<0.05$)

Table 16 **Effect of feeding *Azolla* on the weight gain feed efficiency and protein efficiency in chicks (commercial strain) from 0-4 weeks (Mean ± SE) - Experiment 3**

	% *Azolla* in the ration			
	0	5	10	15
Initial weight (g)	30.7 ± 0.38	31.1 ± 0.42	30.2 ± 0.51	30.0 ± 0.44
Final weight (g)	220.4 ± 3.12	188.3 ± 3.42	176.2 ± 2.85	164.0 ± 2.60
Weight gain (g)	189.7 ± 2.74	157.2 ± 3.21	146.0 ± 2.83	134.0 ± 2.58
Feed intake (g)	456	458	465	472
Feed efficiency (feed/ gain)	2.40	2.89	3.18	3.52
Protein intake (g)	9.15	91.8	93.2	94.4
Protein efficiency ratio	2.07	1.71	1.56	1.42
Mortality %	4.16	4.16	-	-

Table 17 **Effect of feeding *Azolla* on the weight gain feed efficiency and protein efficiency ratio in layer chicks (commercial strain) from 5-8 weeks of age (Mean ± SE) - Experiment 3**

	% *Azolla* in the ration			
	0	5	10	15
Initial weight (g)	220.4 ± 3.12	188.3 ± 3.42	176.2 ± 2.85	164.0 ± 2.60
Final weight (g)	559.6 ± 12.42	512.8 ± 12.93	439.8 ± 13.09	408.6 ± 14.60
Weight gain (g)	339.2 ± 7.51	324.5 ± 6.45	263.6 ± 7.12	244.6 ± 5.47
Feed intake (kg)	1.26	1.27	1.27	1.28
Feed efficiency (feed/ gain)	3.73	3.92	4.83	5.22
Protein intake (g)	253	254	254	256
Protein efficiency ratio	1.34	1.27	1.03	0.95

Table 18 **Effect of feeding *Azolla* on the weight gain feed efficiency and protein efficiency ratio in layer chicks (commercial strain) from 0-8 weeks (Mean ± SE) - Experiment 3**

	% *Azolla* in the ration			
	0	**5**	**10**	**15**
Initial weight (g)	30.7 ± 0.38	31.1 ± 0.42	30.2 ± 0.51	30.0 ± 0.44
Final weight (g)	559.6 ± 12.42	512.8 ± 12.93	439.8 ± 13.09	408.6 ± 14.60
Weight gain (g)	528.9^{b} ± 12.02	481.7^{b} ± 13.29	409.8^{a} ± 14.62	378.6^{a} ± 12.53
Feed intake (kg)	1.72	1.73	1.73	1.75
Feed efficiency (feed/ gain)	3.25	3.59	4.24	4.62
Protein intake (g)	344.5	345.8	347.2	350.4
Protein efficiency ratio	1.53	1.39	1.18	1.08
Mortality %	4.16	4.16	-	-

Mean with different superscript in a row differ significantly ($P < 0.01$)

reduction of nearly 47 gram which would be of economic importance. The weight gain is depicted in a histogram (Fig. 2). The two experiments with chicks clearly suggest that feeding *Azolla* in layer chicks may not be of any advantage. These data contradicted the observations reported earlier by Subudhi and Singh (1978), Castillo *et al.*, (1982) who have reported a beneficial response due to incorporation of *Azolla* in chick mash at 5-10 per cent level. Alcantera and Querubin (1985) have reported comparable performance on 0 and 5 per cent *Azolla* diets, but decreased weight gain at higher levels.

The findings of Querubin *et al.*, (1986) and Taklimi *et al.*, (1993) observed that broiler starter ration may contain upto 15 per cent *Azolla* without any adverse effect once again contradicted the observations made in this study. The reasons for such differences was not obvious. It is perhaps that broiler may be more tolerant than layer chicks for *Azolla* or the difference in strains between these two types of birds or the nature of ingredients between the rations could probably be attributed for the difference in tolerance between layers and broiler chicks.

4.2.4 Effect of feeding *Azolla* on grower performance. (Experiment - 4)

The performance of growers from 9-20 weeks of age on diets containing 0,5, 10 and 15 per cent *Azolla* are given in Tables 19 to 22. The biweekly weight gain from 9-20 weeks is given in Table 19. The perusal of data suggests absence of any noticeable difference between the various treatments except for a marginal reduction in 5 per cent *Azolla* group. The gain was nearly same in the other three groups.

Effect of feeding *Azolla* on weight gain
in Chicks (Commercial) from 0 - 8 weeks
Figure 2

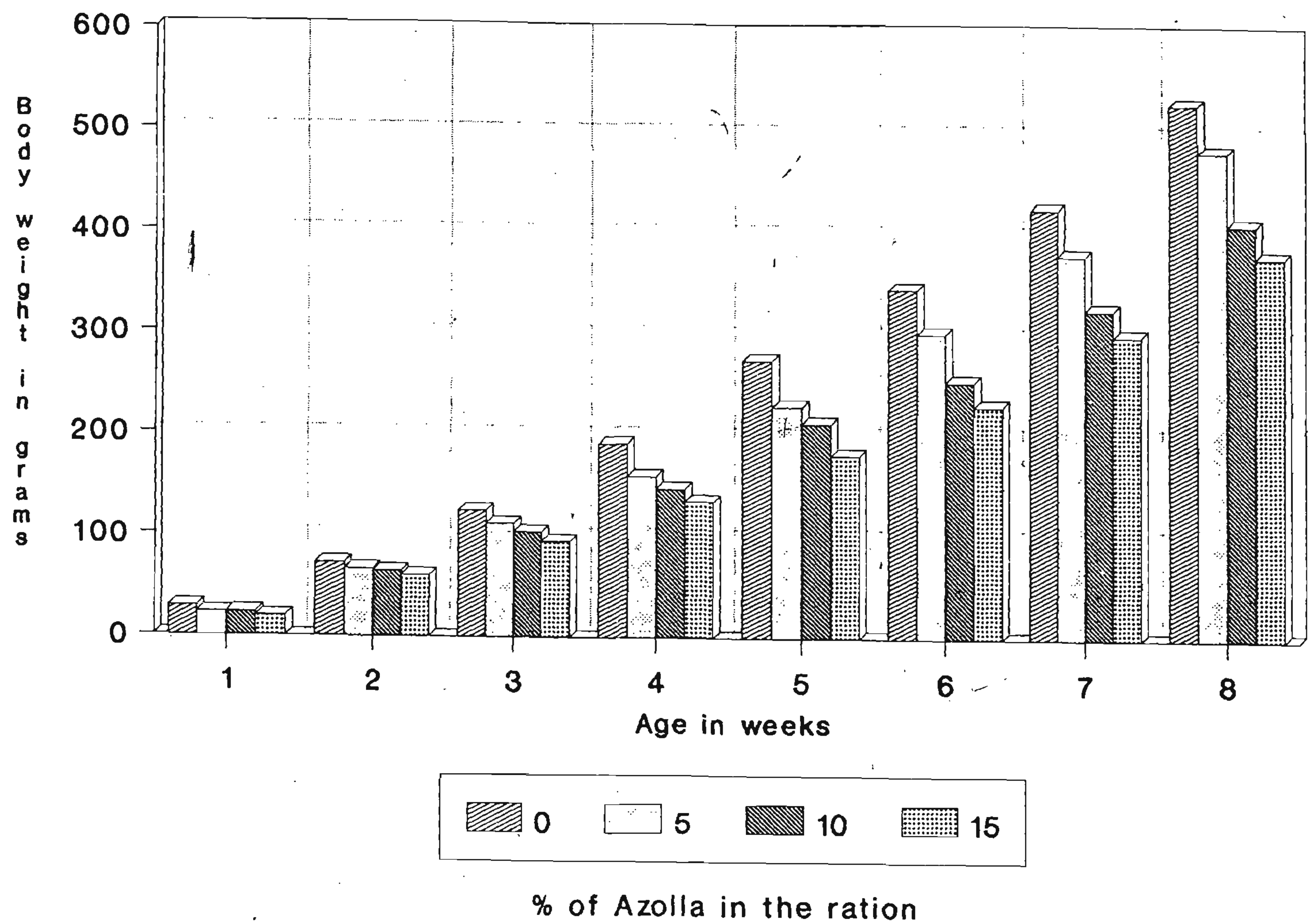


Table 19 **Effect of feeding *Azolla* on bi-weekly body weight gain (g) in WLH growers from 9-20 weeks (Mean* ± SE) - Experiment 4**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
10	151.4 ± 7.41	140.3 ± 6.23	154.6 ± 5.49	152.5 ± 6.58
12	148.2 ± 4.84	137.9 ± 6.94	151.3 ± 4.99	150.2 ± 8.97
14	105.2 ± 5.98	97.9 ± 4.94	108.6 ± 7.98	108.5 ± 6.93
16	96.8 ± 4.59	95.6 ± 6.61	97.1 ± 5.49	98.4 ± 5.53
18	90.0 ± 6.64	89.2 ± 4.73	97.8 ± 4.69	93.1 ± 4.73
20	106.6 ± 5.94	96.4 ± 3.91	101.2 ± 6.99	89.5 ± 6.67
Total gain	698.2^{b} ± 31.12	657.3^{a} ± 24.34	710.7^{b} ± 30.98	692.3^{b} ± 29.03

* Mean with different superscript in a row differ significantly (P<0.01)

The data on biweekly feed intake is given Table 20. The feed intake increased marginally as the level of *Azolla* increased from 0 to 15 per cent. However, the differences were not statistically significant. The particulars of feed efficiency given in Table 21 suggest that inclusion of *Azolla* at all the three levels had an adverse effect on feed efficiency though the decrease was only marginal. The effect of *Azolla* of cumulative performance of growers for the 9-20 weeks of age is given in Table 22. The birds on basal diet gained 698.2 ± 31.12 g as those on 5,10 and 15 per cent *Azolla* diets gained 657.3 ± 24.34, 710.7 ± 30.98 and 692.3 ± 29.03g respectively. The gain on 5 per cent of *Azolla* diet was less whereas in the other three groups the gain was of the same order. However, none of the differences were statistically significant. The feed intake increased marginally from 4.73 to 4.96 kg as the level of *Azolla* increased from 0 to 15 per cent while feed efficiency declined from 6.77 to 7.16 per cent. The age at first egg and weight at maturity were of the same order in all four treatment groups.

The data was suggestive that *Azolla* could be used safely at 15 per cent level in grower rations. The reason for the marginally poor performance on the 5 per cent *Azolla* diet is not clear. The observations made in this study agrees with the report of Subudhi and Singh (1978) who have recorded a significant improvement in weight gain of growers fed 12.5 per cent *Azolla* in the ration.

4.2.5 Effect of feeding *Azolla* on layer performance. (Experiment 5)

The effect of feeding dried *Azolla* meal on layer performance and egg quality for the various 28 day periods from 21 - 40 weeks of age are given in Tables 23 to 35. The cumulative layer performance is given in the Table 23. The birds on 0, 5,

Table 20 Effect of feeding *Azolla* on bi-weekly feed intake (g) in WLH growers from 9-20 weeks - Experiment 4

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
10	630.2	652.7	665.6	672.1
12	658.5	679.1	687.5	701.7
14	725.9	743.7	746.5	756.9
16	831.9	842.9	847.7	859.9
18	868.8	902.4	925.3	939.7
20	1012.9	1018.4	1025.6	1027.6
Total intake	4728.2	4839.2	4898.2	4957.9

Table 21 Effect of feeding *Azolla* on bi-weekly feed efficiency (Feed / gain) in WLH growers from 9-20 weeks - Experiment 4

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
10	4.16	4.65	4.31	4.41
12	4.44	4.93	4.54	4.67
14	6.89	7.59	6.87	6.97
16	8.60	8.81	8.73	8.73
18	9.65	10.24	9.45	10.08
20	9.51	10.56	10.13	11.48

Table 22 **Effect of feeding *Azolla* on the cumulative performance of WLH growers from 9-20 weeks (Mean* ± SE) - Experiment 4**

	% *Azolla* in the ration			
	0	5	10	15
Initial weight (g)	540.6 ± 2.42	541.1 ± 2.61	542.1 ± 2.58	542.6 ± 1.96
Final weight (g)	1238.8 ± 46.34	1198.4 ± 45.56	1252.8 ± 43.18	1234.9^{b} ± 44.30
Weight gain (g)	698.2^{b} ± 31.12	657.3^{a} ± 24.34	710.7^{b} ± 30.98	692.3^{b} ± 29.03
Feed intake (kg)	4.73	4.84	4.90	4.96
Feed efficiency (feed/ gain)	6.77	7.36	6.89	7.16
Age at first egg (days)	133	135	130	136
Weight of first egg (g)	35	32	34	35
Weight at maturity (g)	1225	1200	1232	1230

Mean with different superscripts in a row differ significantly ($P < 0.01$)

Table 23 **Effect of feeding *Azolla* on layer performance and egg quality for the period of 21 - 40 weeks of age Experiment 5**

Parameters	Percentage of *Azolla* in the ration			
	0	5	10	15
Initial weight (g)	1252.8 ± 32.58	1256.4 ± 32.15	1253.0 ± 42.61	1255.4 ± 42.43
Final weight (g)	1645.8 ± 45.14	1628.0 ± 44.55	1617.5 ± 45.89	1600.2 ± 55.75
Weight gain (g)	393^{b} ± 3.12	371.6^{ab} ± 2.87	364.5^{a} ± 2.46	344.8^{a} ± 2.18
Number of eggs laid / bird	109	103	103	110
Kg feed/ dozen eggs	1.64	1.75	1.78	1.71
Kg feed / kg egg mass	2.75	2.91	3.0	2.82
Hen day production %	78.25	75.60	74.86	78.61
Egg weight (g)	50.61 ± 1.08	50.62 ± 0.92	50.18 ± 0.99	50.90 ± 0.82
Shape Index	71.20 ± 0.86	70.86 ± 0.91	71.69 ± 1.28	70.22 ± 1.12
Albumen index	0.085 ± 0.003	0.088 ± 0.0045	0.096 ± 0.0045	0.093 ± 0.0052
Yolk index	0.43 ± 0.0045	0.42 ± 0.0047	0.41 ± 0.0056	0.42 ± 0.0058
Yolk colour ♦	6.22^{a} ± 0.14	6.45^{a} ± 0.15	6.89^{ab} ± 0.14	7.41^{b} ± 0.17
Haugh unit	78.40 ± 6.11	79.57 ± 6.23	80.21 ± 7.11	80.27 ± 7.69
Shell thickness (mm)	0.336 ± 0.008	0.343 ± 0.005	0.341 ± 0.004	0.337 ± 0.005

Values bearing different superscript in a row differ significantly ($P<0.05$).
♦ Roche fan colour

10, and 15 per cent *Azolla* diets gained between 344.8g on 15 per cent *Azolla* diet and 393.0 g on basal diet at 40 weeks of age. The Addition of *Azolla* has progressively decreased the weight gain and the decrease recorded at 10 and 15 per cent levels was significantly different from the basal group. Probably due to difference in the energy level in the diets. The number of eggs laid and the hen day production did not vary much among the different groups. However the feed efficiency for the dozen eggs produced declined marginally from 1.64 to 1.71 with increasing levels of *Azolla*.

The egg quality parameters namely egg weight, shape index, albumen index, yolk index, Haugh unit and egg shell thickness did not vary among the treatment groups. However the pigmenting ability of *Azolla* has been demonstrated clearly as the Roche fan colour score increased with increasing levels of *Azolla* in the diet. The eggs in the control group of birds had a yolk colour score of 6.22 which increased to 6.45, 6.89 and 7.41 on 5, 10 and 15 per cent *Azolla* diets respectively. The colour score at 15 per cent level was significantly different from the control but not from 5 and 10 per cent groups.

The data on body weight for each 28 day periods is given in Table 24. The body weight was maximum on basal diet (1645.8 g) and least on 15 per cent *Azolla* diet (1600.2 g). The feed intake for the five 28 day periods given in Table 25 did not show any variation between the groups and between the periods as well. The data on feed efficiency given in Tables 26 and 27 indicated slight and uniform decline in feed efficiency with increasing levels of *Azolla* at all five 28 day periods. The particulars on Hen day production for the different 28 day periods from 21 - 40 weeks of age are given in Table 28. The per cent hen day production for the first 28 day period from 21 - 24 weeks was 55.15 per cent in the control group

Table 24 **Effect of feeding *Azolla* on body weight (g) in layers for 28 day periods from 21 - 40 weeks of age - (Mean ± SE) Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
20	1252.8 ± 32.58	1256.4 ± 32.15	1253.0 ± 34.61	1255.4 ± 38.43
24	1366.2 ± 42.30	1342.4 ± 32.72	1334.9 ± 32.56	1336.2 ± 32.32
28	1459.8 ± 33.85	1444.8 ± 42.92	1426.8 ± 42.62	1407.5 ± 42.72
32	1515.2 ± 43.41	1507.0 ± 33.24	1495.0 ± 43.17	1472.3 ± 32.92
36	1577.6 ± 34.13	1566.4 ± 44.21	1556.7 ± 33.92	1541.8 ± 43.21
40	1645.8 ± 45.14	1628.0 ± 44.55	1617.5 ± 45.89	1600.2 ± 55.75

Table 25 **Effect of feeding *Azolla* on daily feed intake (g) in layers for 28 day periods from 21 - 40 weeks of age - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	92.7	95.0	96.6	97.8
25 - 28	108.5	111.8	113.6	115.2
29 - 32	110.3	114.6	115.5	116.2
33 - 36	105.2	108.3	110.4	111.3
37 - 40	105.9	109.6	111.0	112.6

Table 26 **Effect of feeding *Azolla* on feed efficiency (kg feed / dozen of eggs) in layers for 28 day periods from 21 - 40 weeks of age - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	2.02	2.15	2.16	2.08
25 - 28	1.45	1.56	1.57	1.53
29 - 32	1.60	1.71	1.75	1.68
33 - 36	1.58	1.68	1.75	1.64
37 - 40	1.53	1.63	1.69	1.61
Mean	1.64	1.75	1.78	1.71

Table 27 Effect of feeding *Azolla* on feed efficiency (kg feed / kg egg mass) in layers for 28 day periods from 21 - 40 weeks of age - Experiment 5

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	3.92	4.05	4.10	3.81
25 - 28	2.50	2.70	2.68	2.61
29 - 32	2.72	2.84	3.02	2.77
33 - 36	2.40	2.58	2.73	2.53
37 - 40	2.19	2.40	2.48	2.40
Mean	2.75	2.91	3.00	2.82

Table 28 Effect of feeding *Azolla* on hen day production (%) in layers for 28 day periods from 21 - 40 weeks of age Experiment 5

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	55.15	53.17	53.77	56.33
25 - 28	90.07	86.23	86.67	90.89
29 - 32	82.72	80.40	79.09	83.24
33 - 36	80.25	77.39	75.73	81.48
37 - 40	83.07	80.81	79.02	81.12
Mean	78.25	75.60	74.80	78.61

reaching a peak of 90 per cent during the second 28 day period and ends up with an average yield of 83 per cent between 37 to 40 weeks. The egg production in 5 and 10 per cent *Azolla* diets were 2 - 3 per cent less as compared to the control group on all periods from 20 - 40 weeks of age. However at 15 per cent level the performance was at par with the control group. The variation in the production performance noted is very small and none of them are statistically significant.

The particulars on egg weight for the 28 day periods are given in the Table 29. The mean egg weight for the four groups varied between 50.18 ± 1.10g in the 10 per cent *Azolla* group to 50.90 ± 1.30g in the 15 per cent *Azolla* group. The egg weight at 24 weeks of age varied between 42.92g in the control group to 45.58g in the 15 per cent *Azolla* group. The egg weight increased in all four treatment groups reaching a maximum of 58.28 ± 0.93 in the control, 56.58 ± 0.92, 56.64 ± 1.39 and 55.92 ± 0.96 on diets containing 5, 10 and 15 per cent *Azolla* respectively. However none of the differences are statistically significant.

The details on shape index (Table 30), albumen index (Table 31), yolk index (Table 32), Haugh unit (Table 33) and shell thickness (Table 34) for the different 28 day periods on diets containing different levels of *Azolla* did not vary significantly between the treatments and between the periods.

The mean shape index was 71.20 ± 1.73, 70.86 ± 1.46, 71.69 ± 1.87 and 70.22 ± 1.83 on diets containing 0, 5, 10 and 15 per cent *Azolla* respectively. The albumin index varied between 0.085 in the control group to 0.096 in the 10 per cent *Azolla* diet. The yolk index varied from 0.41 to 0.43, the Haugh unit between 78.40 to 80.27 and egg shell thickness (mm) between 0.327 to 0.355 in the various

Table 29 **Effect of feeding *Azolla* on egg weight (g) in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	42.92 ± 0.72	44.08 ± 0.90	43.75 ± 0.38	45.58 ± 0.87
25 - 28	48.25 ± 0.81	48.00 ± 0.89	48.83 ± 0.83	48.58 ± 0.80
29 - 32	49.00 ± 1.33	50.25 ± 0.84	48.33 ± 1.00	50.33 ± 0.77
33 - 36	54.58 ± 1.20	54.17 ± 1.06	53.33 ± 1.02	54.08 ± 1.95
37 - 40	58.28 ± 0.93	56.58 ± 0.92	56.64 ± 1.39	55.92 ± 0.96
Mean ± SE	50.61 ± 1.15	50.62 ± 1.03	50.18 ± 1.10	50.90 ± 1.30

Mean values in row do not differ significantly ($P>0.05$)

Table 30 **Effect of feeding *Azolla* on shape index of eggs in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	71.04 ± 0.44	71.60 ± 0.77	73.85 ± 0.73	69.90 ± 1.41
25 - 28	71.24 ± 0.97	69.73 ± 1.02	70.33 ± 0.67	70.79 ± 1.35
29 - 32	70.98 ± 0.68	71.28 ± 0.71	72.59 ± 0.89	70.41 ± 0.86
33 - 36	72.65 ± 2.65	71.24 ± 2.20	70.87 ± 2.70	70.75 ± 2.08
37 - 40	70.11 ± 1.82	70.45 ± 1.23	70.80 ± 2.22	69.23 ± 2.13
Mean ± SE	71.20 ± 1.73	70.86 ± 1.46	71.69 ± 1.87	70.22 ± 1.83

Table 31 **Effect of feeding *Azolla* on albumen index of eggs in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	0.073 ± 0.0031	0.081 ± 0.0034	0.079 ± 0.0043	0.083 ± 0.0055
25 - 28	0.071 ± 0.0024	0.072 ± 0.0044	0.082 ± 0.0043	0.074 ± 0.0051
29 - 32	0.092 ± 0.0011	0.106 ± 0.0038	0.106 ± 0.0049	0.108 ± 0.0052
33 - 36	0.104 ± 0.0041	0.097 ± 0.0047	0.117 ± 0.0050	0.108 ± 0.0056
37 - 40	0.085 ± 0.0033	0.082 ± 0.0032	0.098 ± 0.0047	0.093 ± 0.0045
Mean ± SE	0.085 ± 0.0029	0.088 ± 0.0044	0.096 ± 0.0052	0.093 ± 0.0058

Table 32 Effect of feeding *Azolla* on yolk index of eggs in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 5

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	0.45 ± 0.0029	0.42 ± 0.0032	0.39 ± 0.0021	0.42 ± 0.0052
25 - 28	0.44 ± 0.0031	0.44 ± 0.0062	0.43 ± 0.0042	0.42 ± 0.0057
29 - 32	0.40 ± 0.0062	0.41 ± 0.0043	0.40 ± 0.0036	0.41 ± 0.0064
33 - 36	0.43 ± 0.0044	0.42 ± 0.0047	0.43 ± 0.0061	0.44 ± 0.0045
37 - 40	0.43 ± 0.0038	0.41 ± 0.0056	0.42 ± 0.0054	0.43 ± 0.0053
Mean ± SE	0.43 ± 0.0046	0.42 ± 0.0055	0.41 ± 0.0050	0.42 ± 0.0060

Table 33 **Effect of feeding *Azolla* on Haugh units of eggs in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	76.42 ± 7.63	81.17 ± 5.96	81.67 ± 4.39	82.83 ± 7.45
25 - 28	78.58 ± 5.89	79.00 ± 5.00	83.00 ± 5.05	81.58 ± 9.03
29 - 32	79.33 ± 5.02	78.33 ± 4.92	78.16 ± 6.34	79.50 ± 5.63
33 - 36	79.25 ± 9.58	79.75 ± 8.19	80.08 ± 9.36	80.00 ± 9.24
37 - 40	78.42 ± 6.85	79.58 ± 4.69	78.16 ± 5.14	77.42 ± 7.81
Mean ± SE	78.40 ± 8.01	79.57 ± 5.90	80.21 ± 6.31	80.27 ± 8.88

Table 34 **Effect of feeding *Azolla* on egg shell thickness (mm) in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 5**

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	0.341 ± 0.009	0.351 ± 0.005	0.342 ± 0.005	0.334 ± 0.004
25 - 28	0.346 ± 0.006	0.334 ± 0.006	0.341 ± 0.004	0.335 ± 0.005
29 - 32	0.332 ± 0.005	0.341 ± 0.004	0.332 ± 0.003	0.336 ± 0.005
33 - 36	0.332 ± 0.005	0.335 ± 0.006	0.335 ± 0.005	0.331 ± 0.004
37 - 40	0.332 ± 0.005	0.335 ± 0.006	0.335 ± 0.005	0.331 ± 0.004
Mean ± SE	0.327 ± 0.006	0.353 ± 0.006	0.355 ± 0.005	0.351 ± 0.005

treatment groups. The differences were small and has no significance with reference to the dietary treatments.

The yolk colour score has been influenced by the level of *Azolla* in the ration for the different 28 day periods from 21 - 40 weeks of age and the details are furnished in Table 35. The Roche colour score for the control diet at 24 weeks of age was 6.17. The score at 28, 32, 36 and 40 weeks of age was 6.33, 6.00, 6.17 and 6.42 respectively with a mean score of 6.22 for the control group for all five 28 day periods. The score for the 5 per cent *Azolla* diet varied from 6.17 to 6.83 with a mean value of 6.45. The score for the 10 per cent group varied from 6.50 at 24 weeks age to 7.83 at 40 weeks with a mean of 6.89 ± 0.16. The 15 per cent *Azolla* group had a much higher pigmenting ability as shown by the score which varied from 7.00 ± 0.21 at 24 weeks of age to 8.33 ± 0.16 at 40 weeks of age with a mean of 7.41 ± 0.19.

The data clearly showed that *Azolla* has the ability to pigment the yolk and the score at 15 per cent level is significantly different from the lower levels of *Azolla*. Similar influence on yolk colour and an adverse effect on weight gain due to inclusion of *Azolla* in layer ration was reported by Bastian (1987). Thus the data indicate that *Azolla* can be included in layer rations upto 15 per cent level with economic returns even though there may be an marginal reduction in production efficiency, but in terms of return over the feed cost *Azolla* may be an useful feed at 15 per cent level.

Table 35 Effect of feeding *Azolla* on yolk colour in layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) Experiment 5

Age in weeks	% *Azolla* in the ration			
	0	5	10	15
21 - 24	6.17 ± 0.11	6.50 ± 0.19	6.50 ± 0.15	7.00 ± 0.21
25 - 28	6.33^{a} ± 0.14	6.42^{a} ± 0.19	6.67^{ab} ± 0.14	7.08^{ab} ± 0.19
29 - 32	6.00^{a} ± 0.20	6.17^{a} ± 0.11	6.62^{ab} ± 0.15	7.22^{a} ± 0.14
33 - 36	6.17^{a} ± 0.14	6.33^{a} ± 0.16	6.83^{ab} ± 0.14	7.42^{b} ± 0.12
37 - 40	6.42^{a} ± 0.12	6.83^{a} ± 0.14	7.83^{b} ± 0.13	8.33^{c} ± 0.16
Mean ± SE	6.22^{a} ± 0.16	6.45^{a} ± 0.16	6.89^{ab} ± 0.16	7.41^{b} ± 0.19

4.2.5.1 Histopathological examination

4.2.5.1.1 Gross pathology

Complete necropsy was done on four birds each in control and 15 per cent *Azolla* groups. There was no appreciable change in the gross pathology of the different organs in the control group. However, the liver in two of the 15 per cent *Azolla* group was slightly enlarged and mottled. The kidney was also mildly congested in these birds. The mucosa of small intestine revealed areas of congestion.

4.2.5.1.2 Histopathology

The birds in the control group were free from any distinct lesions. Histopathological examination of four birds from 15 per cent *Azolla* group sacrificed at the end of 40th week showed marked congestions of lungs and kidney. The liver revealed congestion and distention of sinusoids and diffused vascular degenerative changes in hepatocytes (Plate 2). In the small intestine there was a mild infiltration of mononuclear cells in the lamina propria. The lesions observed could possibly be responsible for the reduction in feed efficiency in rations containing *Azolla* as described earlier. At the same time the lesions may not be severe enough to cause any major ill effects on the birds performance, health and livability.

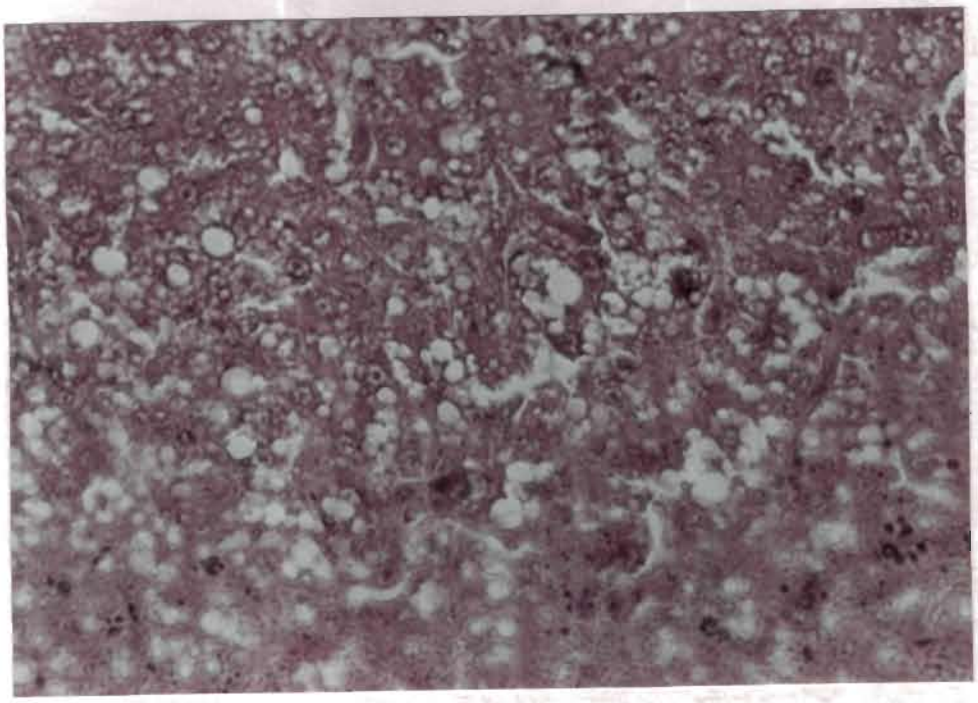
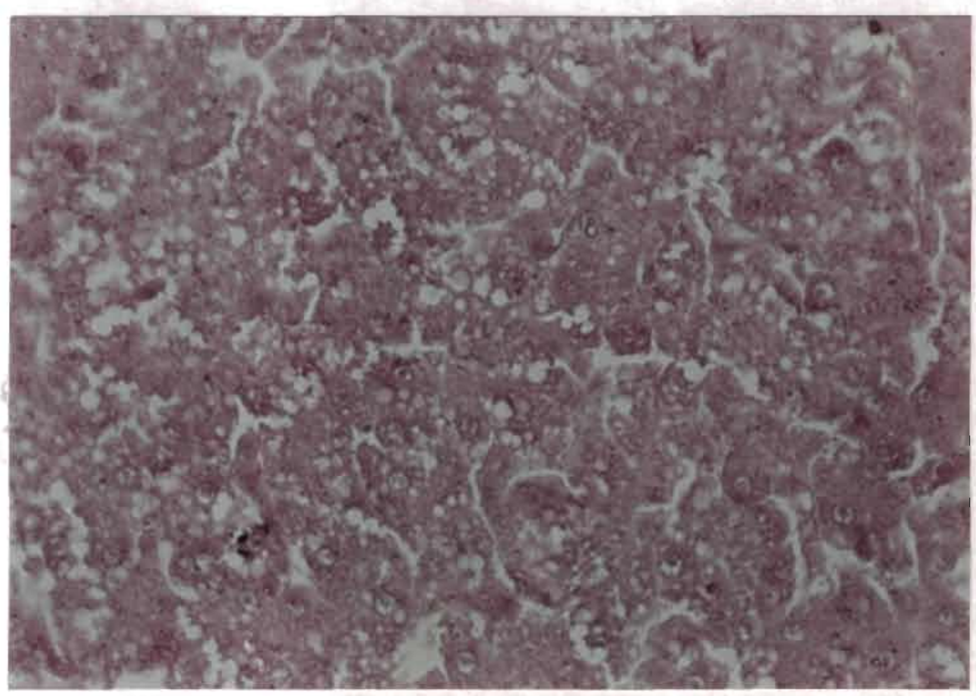


Plate 2 - Distention of sinusoids and diffused vascular degenerative changes in heptocytes (H & E 528)



4.2.6 Effect of feeding *Azolla* on performance and egg quality in caged layers (Experiment 6)

The performance particulars of layers relating to weight gain, feed efficiency and egg quality on diets containing *Azolla* at 0, 15, 20 and 25 per cent levels in caged layers during 21 - 40 weeks of age are given in Tables 36 - 48. The cumulative performance at 40 weeks of age shown in Table 36 concurred with the observations made in the previous experiment. The weight gain in the control group of birds was 405.9 ± 5.42, which decreased progressively to 395.6 ± 4.12, 351.2 ± 5.58 and 316.0 ± 6.12 with increasing levels of *Azolla* at 15, 20, and 25 per cent in the ration. The differences between 20 and 25 per cent groups as compared to 0 and 15 per cent groups were statistically significant at $P<0.05$. The weight gain for the different 28 day periods is given in Table 37.

The production of eggs decreased from 112 to 96 eggs from the basal to 25 per cent *Azolla* diet while the feed efficiency declined from 1.73 to 2.04 with increasing levels of *Azolla*. The particulars on feed intake, and feed efficiencies for the 28 day periods are given in Table 38, 39 and 40. The Hen day production particulars are furnished in Table 41. A marginal reduction in egg weight was noticed at higher level of *Azolla* feeding (53.02 g in the control group Vs 51.52 in the 25 per cent *Azolla* group) (Table 42).

The shape index (Table 43), albumen index (Table 44), yolk index (Table 45), Haugh unit (Table 46) and shell thickness (Table 47) did not vary among the treatments as in the previous feeding trial.

Table 36 **Effect of feeding *Azolla* on layer performance and egg Quality in caged layers for the period from 21-40 weeks of age (Mean ± SE)**

Parameters	% *Azolla* in the ration			
	0	15	20	25
Initial weight (g)	1252.8 ± 30.58	1260.1 ± 41.22	1261.1 ± 35.75	1260.6 ± 37.88
Final weight (g)	1658.6^{c} ± 43.31	1655.7^{c} ± 33.96	1612.3^{b} ± 42.64	1576.6^{a} ± 41.97
Weight gain (g)	405.9^{c} ± 5.42	395.6^{c} ± 4.12	351.2^{b} ± 5.58	316.0^{a} ± 6.12
Number of eggs laid/Bird	112	112	105	96
Feed / dozen eggs	1.73	1.73	1.90	2.04
Feed / kg egg mass	2.73	2.72	3.08	3.30
Hen day production %	80.12	81.47	74.84	69.86
Egg weight (g)	53.02 ± 3.31	53.15 ± 4.54	51.67 ± 4.87	51.52 ± 4.90
Shape index	72.55 ± 2.12	73.31 ± 5.41	73.34 ± 4.62	72.60 ± 2.44
Albumen index	0.114 ± 0.002	0.118 ± 0.004	0.116 ± 0.004	0.117 ± 0.003
Yolk colour♦	6.30^{a} ± 0.18	7.45^{b} ± 0.22	7.77^{c} ± 0.32	7.80^{c} ± 0.38
Yolk index	0.428 ± 0.002	0.428 ± 0.002	0.423 ± 0.002	0.417 ± 0.003
Haugh unit	81.34 ± 5.38	79.42 ± 6.12	79.77 ± 5.20	79.78 ± 6.54
Shell thickness (mm)	0.359 ± 0.0006	0.347 ± 0.006	0.353 ± 0.007	0.352 ± 0.005

Mean with defferent superscripts in a row differ significantly ($P<0.05$).

♦ Roche colour score

Table 37 **Effect of feeding *Azolla* on body weight (g) in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
20	1252.8 ± 30.58	1260.8 ± 41.22	1261.1 ± 35.75	1260.6 ± 37.88
24	1366.2 ± 31.24	1365.6 ± 40.28	1331.1 ± 41.12	1320.0 ± 40.15
28	1465.3 ± 34.62	1460.6 ± 35.56	1432.5 ± 38.56	1419.7 ± 38.57
32	1542.8^{b} ± 38.54	1529.4^{b} ± 36.74	1485.6^{a} ± 40.15	1462.9^{a} ± 39.46
36	1608.1^{b} ± 31.46	1607.1^{b} ± 38.29	1539.2^{a} ± 37.46	1521.4^{a} ± 32.14
40	1658.6^{b} ± 43.31	1655.7^{a} ± 33.96	1612.3^{ab} ± 47.64	1576.6^{a} ± 57.97

Mean with different superscripts in a row differ significantly ($P<0.05$)

Table 38 **Effect of feeding *Azolla* on the mean daily feed intake (g) in caged layers for 28 day periods from 21 - 40 weeks of age (Mean) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	97.8	98.9	100.6	102.1
25 - 28	120.6	121.8	124.0	125.1
29 - 32	120.5	122.3	123.9	125.1
33 - 36	113.1	115.1	117.7	118.5
37 - 40	114.5	116.8	118.5	119.6
Mean	111.3	115.0	116.9	118.1

Table 39 **Effect of feeding *Azolla* on the average feed efficiency (kg feed / dozen eggs) in caged layers - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	2.08	2.07	2.18	2.30
25 - 28	1.57	1.57	1.75	1.87
29 - 32	1.70	1.70	1.86	1.98
33 - 36	1.66	1.66	1.88	2.04
37 - 40	1.62	1.63	1.82	2.01
Mean	1.73	1.73	1.90	2.04

Table 40 Effect of feeding *Azolla* on feed efficiency (kg feed / kg egg mass) in caged layers - Experiment 6

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	3.54	3.51	3.83	4.03
25 - 28	2.54	2.67	2.94	2.99
29 - 32	2.60	2.67	2.90	3.20
33 - 36	2.58	2.37	2.84	3.02
37 - 40	2.37	2.38	2.87	3.25
Mean	2.73	2.72	3.08	3.30

Table 41 Effect of feeding *Azolla* on the average hen day production (%) in caged layers for 28 day periods from 21 - 40 weeks of age - Experiment 6

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	56.65	57.44	55.36	53.47
25 - 28	92.16	93.45	85.22	80.26
29 - 32	85.22	86.68	80.26	74.23
33 - 36	81.95	83.47	75.00	69.91
37 - 40	84.62	86.33	78.37	71.43
Mean	80.12	81.47	74.84	69.86

Table 42 **Effect of feeding *Azolla* on egg weight (g) in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	48.67 ± 1.11	49.08 ± 0.83	47.50 ± 0.81	47.42 ± 0.75
25 - 28	51.50 ± 5.17	48.83 ± 4.42	49.50 ± 5.89	52.08 ± 6.42
29 - 32	54.42 ± 2.77	52.83 ± 6.39	53.33 ± 3.05	51.33 ± 4.73
33 - 36	53.50 ± 2.53	58.08 ± 2.57	55.33 ± 4.35	56.08 ± 4.06
37 - 40	57.00 ± 3.56	56.92 ± 5.94	52.67 ± 7.43	50.67 ± 6.27
Mean ± SE	53.02 ± 3.31	53.15 ± 4.54	51.67 ± 4.87	51.52 ± 4.90

Table 43 Effect of feeding *Azolla* on shape index of eggs in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	73.87 ± 1.63	71.02 ± 2.64	73.25 ± 2.34	72.27 ± 1.56
25 - 28	70.83 ± 2.83	72.04 ± 3.41	75.75 ± 11.73	74.26 ± 2.27
29 - 32	73.24 ± 1.41	77.39 ± 12.05	71.50 ± 0.97	72.30 ± 2.01
33 - 36	72.72 ± 1.30	73.15 ± 14.53	71.47 ± 3.53	72.08 ± 2.61
37 - 40	72.07 ± 3.28	72.95 ± 2.21	74.74 ± 3.48	72.07 ± 3.54
Mean ± SE	72.55 ± 2.92	73.31 ± 5.41	73.34 ± 4.62	72.60 ± 2.44

Table 44 Effect of feeding *Azolla* on Albumen index of eggs in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	0.134 ± 0.001	0.132 ± 0.002	0.130 ± 0.002	0.130 ± 0.002
25 - 28	0.120 ± 0.001	0.131 ± 0.001	0.121 ± 0.001	0.126 ± 0.002
29 - 32	0.109 ± 0.003	0.119 ± 0.008	0.118 ± 0.006	0.112 ± 0.001
33 - 36	0.111 ± 0.002	0.109 ± 0.001	0.118 ± 0.001	0.116 ± 0.004
37 - 40	0.097 ± 0.003	0.098 ± 0.001	0.094 ± 0.005	0.100 ± 0.003
Mean ± SE	0.114 ± 0.002	0.118 ± 0.004	0.116 ± 0.004	0.117 ± 0.003

Table 45 **Effect of feeding *Azolla* on Yolk index of eggs in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	0.450 ± 0.001	0.447 ± 0.002	0.450 ± 0.001	0.438 ± 0.002
25 - 28	0.451 ± 0.001	0.450 ± 0.001	0.450 ± 0.002	0.449 ± 0.001
29 - 32	0.429 ± 0.002	0.430 ± 0.002	0.431 ± 0.003	0.424 ± 0.001
33 - 36	0.427 ± 0.004	0.421 ± 0.001	0.420 ± 0.002	0.412 ± 0.003
37 - 40	0.383 ± 0.001	0.394 ± 0.002	0.365 ± 0.001	0.362 ± 0.006
Mean ± SE	0.428 ± 0.002	0.428 ± 0.002	0.423 ± 0.002	0.417 ± 0.003

Table 46 **Effect of feeding *Azolla* on Haugh units of eggs in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	81.42 ± 7.90	80.50 ± 9.36	79.17 ± 8.48	81.08 ± 7.05
25 - 28	81.83 ± 4.90	79.50 ± 2.29	80.42 ± 2.52	79.42 ± 7.96
29 - 32	81.35 ± 3.72	79.75 ± 4.72	77.83 ± 5.19	77.42 ± 8.15
33 - 36	79.50 ± 6.09	75.83 ± 6.63	80.91 ± 3.73	80.25 ± 9.40
37 - 40	82.58 ± 2.76	81.50 ± 5.32	80.50 ± 4.02	80.75 ± 5.00
Mean ± SE	81.34 ± 5.38	79.42 ± 6.12	79.77 ± 5.20	79.78 ± 6.54

Table 47 **Effect of feeding *Azolla* on egg shell thickness (mm) in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6**

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	0.393 ± 0.008	0.348 ± 0.008	0.366 ± 0.011	0.353 ± 0.005
25 - 28	0.341 ± 0.005	0.339 ± 0.006	0.348 ± 0.007	0.354 ± 0.005
29 - 32	0.357 ± 0.004	0.340 ± 0.005	0.344 ± 0.003	0.347 ± 0.004
33 - 36	0.339 ± 0.008	0.353 ± 0.007	0.353 ± 0.010	0.352 ± 0.007
37 - 40	0.363 ± 0.006	0.354 ± 0.005	0.355 ± 0.004	0.354 ± 0.004
Mean ± SE	0.359 ± 0.006	0.347 ± 0.006	0.353 ± 0.007	0.352 ± 0.005

The yolk colour improved significantly due to the addition of *Azolla*, confirming the observations made in the previous experiment. The Roche colour score (Table 48) was 6.30 ± 0.18 for the control diet as compared to 7.80 ± 0.38 for the 25 per cent *Azolla* diet. The addition of *Azolla* has significantly improved the yolk colour in all rations as compared to control group (Plate 3).

The details on the various 28 day periods performance of feed intake, feed efficiency and egg quality parameters are given in Tables 37 - 48. The feed efficiency declined with increasing levels of *Azolla* and so also the per cent hen day production. The egg quality parameters such as shape index, albumen index, yolk index, Haugh unit and shell thickness did not vary significantly between the treatments.

The data is suggestive that *Azolla* could be useful in layer ration upto 15 per cent level as observed in earlier experiment but not at higher levels beyond 15 per cent. But Bastian (1987) had reported significant weight reduction beyond *Azolla* at 15 per cent level. The low energy content of *Azolla* and possible aminoacid deficiencies at higher level of incorporation may be possible reasons for a poor performance at 20 and 25 per cent levels. Parthasarathy (1995) has shown that the protein quality of *Azolla* will be improved by the inclusion of 0.3 per cent lysine and 0.2 per cent methionine in broiler starter rations. Thus it is possible that at levels beyond 15 per-cent some of these amino acids may be limiting, affecting the overall production of layers.

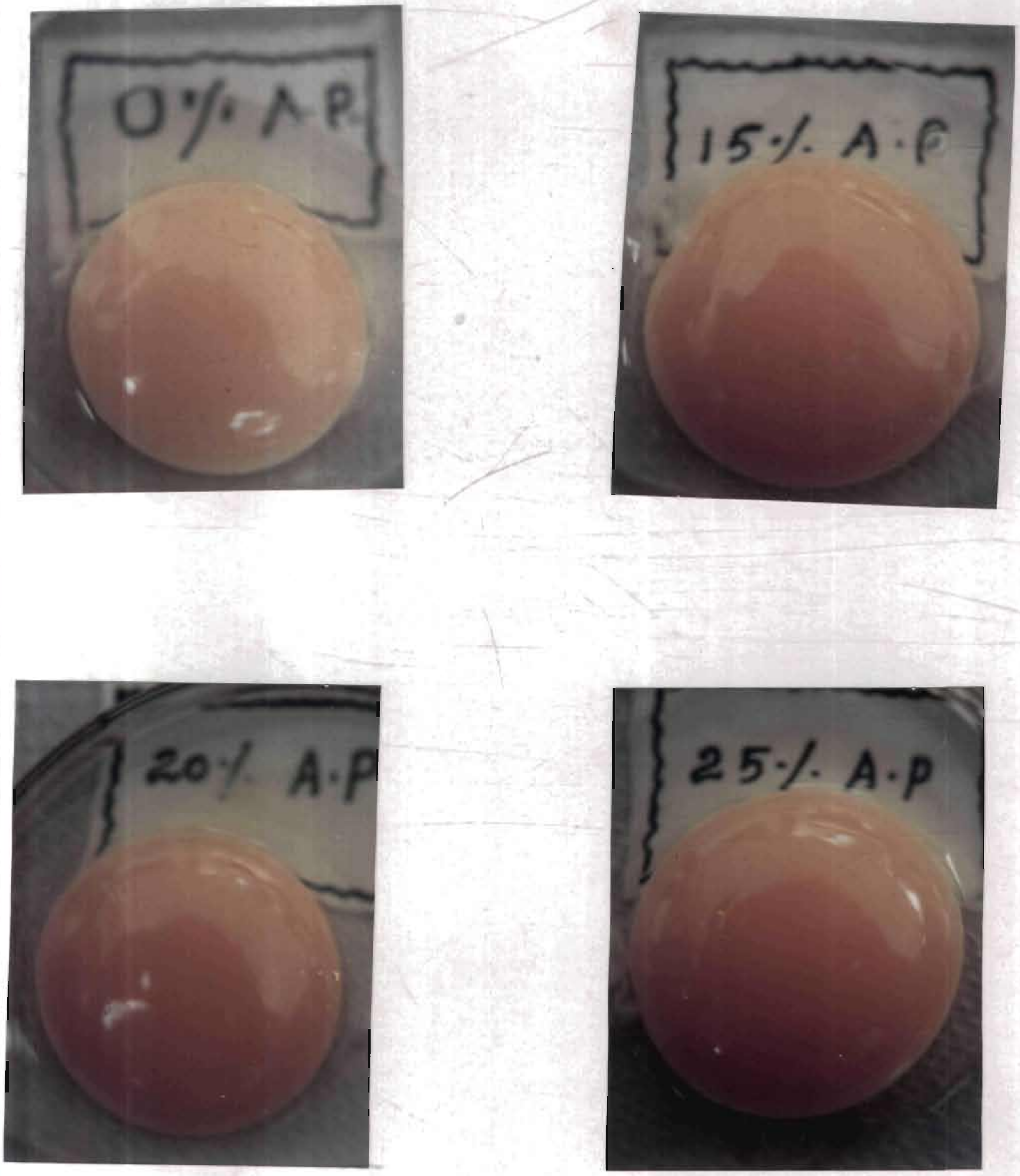


Plate 3 - Pigmentation of yolk with varying level of *Azolla* in the diet

Table 48 Effect of feeding *Azolla* on Yolk colour♦ of eggs in caged layers for 28 day periods from 21 - 40 weeks of age (Mean ± SE) - Experiment 6

Age in weeks	% *Azolla* in the ration			
	0	15	20	25
21 - 24	$6.25^{a} \pm 0.25$	$7.08^{b} \pm 0.17$	$7.08^{b} \pm 0.17$	$7.58^{b} \pm 0.37$
25 - 28	$6.08^{a} \pm 0.12$	$7.17^{b} \pm 0.18$	$7.67^{b} \pm 0.30$	$7.33^{b} \pm 0.40$
29 - 32	$6.33^{a} \pm 0.16$	$7.42^{b} \pm 0.17$	$7.33^{b} \pm 0.55$	$7.66^{b} \pm 0.50$
33 - 36	$6.38^{a} \pm 0.22$	$7.83^{b} \pm 0.20$	$8.25^{b} \pm 0.25$	$8.17^{b} \pm 0.14$
37 - 40	$6.48^{a} \pm 0.11$	$7.75^{b} \pm 0.34$	$8.00^{b} \pm 0.19$	$8.25^{c} \pm 0.38$
Mean ± SE	$6.30^{a} \pm 0.18$	$7.45^{b} \pm 0.22$	$7.77^{c} \pm 0.32$	$7.80^{c} \pm 0.38$

Mean with different superscript in a row differ significantly ($P<0.05$)
♦ Roche colour score

4.3 Nitrogen balance study in layers at 40 weeks of age

The data on nitrogen balance in layers at 40 weeks of age as influenced by *Azolla* feeding is given in Table 49. The layers on basal diet had positive balance with a retention of 56.65 per cent nitrogen over the three day experimental period. The nitrogen balance decreased marginally to 53.89, 51.69 and 50.00 per cent on 15, 20 and 25 per cent *Azolla* diets. The reduction though marginal could be one of the reason for decreasing the weight gain with increasing levels of *Azolla*.

Bastian (1987) reported better protein digestibility with *Azolla microphylla* but not with *Azolla pinnata*. Hence it is possible that strain difference like species difference, may be responsible for the variations in their utilisation.

4.4 Economics

The returns over feed cost is given in Table 50 for the layer trial in deep litter houses and in Table 51 for caged layer study. In experiment 5 the returns over feed cost in the control group work out to the Rs. 16.30 and in 5, 10 and 15 per cent *Azolla* diet the returns are Rs. 10.90, 14.25 and 23.85 respectively. Similarly in the second layer trial the returns over the feed cost worked out to the Rs. 11.00, 22.65, 25.40 and 20.10 for 0, 15, 20 and 25 per cent respectively. The returns are better than control in 15, 20 and 25 per cent *Azolla* diets.

Table 49 Effect of feeding *Azolla* on per cent nitrogen retention in layers (Mean ± SE)

	% *Azolla* in the ration			
	0	15	20	25
Initial body weight (g)	1298.3	1300.0	1299.1	1301.6
Final body weight (g)	1309.4	1308.4	1306.8	1308.2
Nitrogen intake (g) day	3.16	3.21	3.25	3.28
Nitrogen outgo (g) / day	1.37	1.48	1.57	1.64
Nitrogen retained (g)/day	1.79	1.73	1.68	1.64
Nitrogen retained (%)	56.65	53.89	51.69	50.0

Table 50 **Economics of feeding *Azolla* in layers from 21 to 40 weeks (Deep litter system)**

Criteria	% *Azolla* in the ration			
	0	5	10	15
Cost of feed / kg (Rs)	6.35	6 - 10	5.80	5.56
Total feed consumed / Bird (kg)	14.60	15.1	15.3	15.5
Total feed cost (Rs)	92.70	92.10	88.75	86.20
Number of eggs laid/Bird	109	103	103	110
Cost of eggs @ Rs.1/egg	109	103	103	110
Returns over feed cost (Rs)	16.30	10.90	14.25	23.80

Table 51 **Economics of feeding *Azolla* in layers from 21 to 40 weeks (Cage system)**

Criteria	% *Azolla* in the ration			
	0	15	20	25
Cost of feed / kg (Rs)	6.35	5.55	4.85	4.60
Total feed consumed / Bird (kg)	15.9	16.1	16.4	16.5
Total feed cost (Rs)	101.00	89.35	79.54	75.90
Number of eggs laid/Bird	112	112	105	96
Cost of eggs @ Rs.1/egg	112	112	105	96
Returns over feed cost (Rs)	11.00	22.65	25.46	20.10

Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSIONS

The water fern, *Azolla pinnata,* was evaluated for its potential as a feedstuff in poultry rations by chemical analysis and biological experimentation.

Samples of *Azolla pinnata* from four different locations in Tamil Nadu, namely Rice Research Institute, Aduthurai, Blue-green algae project, Aduthurai, Tamil Nadu Agricultural University, Coimbatore and Madurai campus were collected and chemically evaluated.

Fresh *Azolla* had a mean dry matter content of 12.16 ± 1.21 per cent. The sundried *Azolla* contained (per cent DMB) : crude protein - 25.46 ± 0.69, crude fibre - 12.46 ± 0.55, ether extractives - 1.88 ± 0.11, total ash - 15.06 ± 1.03, nitrogen free extract - 45.14 ± 0.44 and acid insoluble ash 6.80 ± 0.60. The variations among the samples analysed were small except for crude protein and total ash. The sample from the blue-green algae project had least amount of proteins (21.99 ± 0.57), while the sample from the Rice Research Institute had the highest (27.50 ± 0.54). The total ash and acid insoluble ash contents were high in the sample collected from Tamil Nadu Agricultural University, Coimbatore.

The mean values for neutral detergent fibre, acid detergent fibre, cellulose, hemicellulose and lignin were 42.39 ± 0.38, 27.94 ± 0.17, 12.44 ± 0.09, 14.45 ± 0.09 and 6.93 ± 0.07 per cent respectively.

Azolla contained (in per cent) calcium 1.48, phosphorus 0.64, magnesium 0.78, sodium 0.16 and potassium 2.56. The concentration of iron, copper, manganese, zinc and cobalt was 1321, 15.17, 356.5, 49.80, and 6.95 mg / kg dry *Azolla* respectively.

The *Azolla pinnata* contained (per cent protein) higher levels of Arginine - 6.73, leucine - 8.37, valine - 5.01, isoleucine - 5.20, but a low level of methionine (1.09).

The biological experimentations included one metabolizable energy determination trial using 12 roosters of 20 weeks of age, two trials in chicks from 0-8 weeks of age using 96 chicks each, one trial with 184 growers from 9 - 20 weeks of age, and two trials in layers using 200 and 144 birds.

Sundried *Azolla* contained 1659 kcal of TME per kilogram which will make it as a useful replacement for low energy feeds like wheat bran or rice bran.

The feeding trials with day old chicks fed *Azolla* at 0, 5, 10, and 15 per cent levels, replacing a protein mix of groundnut cake and wheat bran (50:50), resulted in a gain of (trial 1) 513.5, 494.8, 461.1, 406.3 g respectively. The feed intake increased marginally while the feed efficiency declined progressively with increasing levels of *Azolla*. The decrease in weight gain at 10 and 15 per cent levels was statistically significant ($P<0.05$) as compared to control and 5 per cent *Azolla* diet.

In the second trial feeding of sundried *Azolla* to a commercial hybrid strain at 0, 5, 10 and 15 per cent levels resulted in a gain of 528.9 ± 12.02, 481.7 ± 13.29, 409.8 ± 14.62, 378.6 ± 12.53 g respectively. As in previous trial reduction in weight gain on 10 and 15 per cent *Azolla* diets were statistically significant ($P<0.05$) as compared to 0 and 5 per cent *Azolla* diets. However inclusion of *Azolla* did not influence the feed intake and protein intake drastically.

The third trial with growers from 9 - 20 weeks of age feeding 0, 5, 10 and 15 per cent *Azolla* containing diets resulted in a gain of 698.2, 657.3, 710.7 and 692.3g respectively. The reduced gain on five per cent *Azolla* diet was significantly less ($P<0.05$) from the rest of the groups. The feed intake, feed efficiency and weight at maturity did not vary between the treatments.

In the first layer experiment birds receiving *Azolla* at 0, 5, 10 and 15 per cent levels during the 20 to 40 weeks of age gained 393.0, 371.6, 364.5 and 344.8g respectively at 40 weeks of age. The birds on basal diet gained significantly more as compared to 10 and 15 per cent *Azolla* group. The feed intake and feed efficiency did not vary among the treatments. The egg quality parameters except for yolk colour did not vary between the treatments. The Roche fan colour score for egg yolk from 0, 15, 10 and 15 per cent *Azolla* based diets was 6.22, 6.45, 6.81 and 7.41 respectively.

Histopathological examination of four birds from the 15 per cent *Azolla* group revealed, mild congestion of lungs and liver with distention of sinusoids and diffused vascular degenerative changes in the heptocytes.

In caged layer experiment *Azolla* was included at higher levels namely 0, 15, 20 and 25 per cent. The weight gain in the control group of birds was 405g which decreased progressively to 395.6, 259.2 and 316 g respectively with increasing levels of *Azolla* from 0, 15, 20 and 25 per cent. A decreased weight gain on 20 and 25 per cent *Azolla* groups as compared to 0 and 15 per cent were significantly different at 5 per cent level.

As in earlier layer experiment the feed intake increased with increasing levels of *Azolla* while the feed and protein efficiency decreased progressively. The egg quality parameters except for yolk colour were of the same order in all treatments.

A balance trial conducted at the end of 40 weeks of age revealed a positive nitrogen balance with a retention of 56.65 per cent nitrogen in the basal group. The nitrogen balance decreased marginally to 53.89, 51.69 and 50.00 per cent on 15, 20 and 25 per cent *Azolla* diets.

The inclusion of *Azolla* in layer rations had resulted in a net return over feed cost of Rs. 16.30, 10.90, 14.25 and 23.85 on 0, 5, 10 and 15 per cent *Azolla* diets. The return over feed cost on 0, 15, 20 and 25 per cent *Azolla* diet was Rs. 11, 22.65, 25.40 and 20.10 respectively.

Conclusions

Fresh *Azolla* is low in dry matter and consequently low in nutrient density.

Sundried *Azolla* is potentially useful to replace feeds low in energy and moderately high in protein. The wider calcium - phosphorus ratio and low level of methionine might limit its usefulness unless corrected by supplementation.

Sundried *Azolla* may be included in chick starter ration at 5 per cent level, inspite of a marginal reduction in weight gain, if such inclusion proves economical after conducting few large scale field trials. In grower and layer rations *Azolla* can be included at 15 per cent level without any adverse effect on growth, feed efficiency and egg quality. Inclusion at higher levels in layer rations decreases the layers performance. The nitrogen retention and egg productions decreased at increasing levels of *Azolla* from 15 to 25 per cent. *Azolla* will be useful in pigmenting the yolk at all levels of inclusions.

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