Review of Literature
II. REVIEW OF LITERATURE

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Inadequate availability of good quality feed is regarded as a major constraint to the prevalent poultry production system. The conventional feed ingredients, particularly protein supplements are expensive and are not always available at affordable prices. The use of animal protein feed ingredients in poultry diet is becoming increasingly expensive. This leads to a search for cheap and easily available alternate protein sources. Aquatic plants have long been used in many developing countries as a feed source for livestock and poultry.

2.1 Aquatic and other plants in animal feeding

Recently there is an increased emphasis on the use of aquatic plants in poultry rations because the protein and other nutrient content in them are comparable to certain leguminous plants (Table: 2.1).

Table 2.1: Chemical composition (% DM basis) of aquatic plants and other plants (Muztar et al., 1976):

<table>
<thead>
<tr>
<th>Plantspecies</th>
<th>CP</th>
<th>CF</th>
<th>EE</th>
<th>TA</th>
<th>Ca</th>
<th>P</th>
<th>GE kcal/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>17.70</td>
<td>24.20</td>
<td>3.65</td>
<td>9.90</td>
<td>1.90</td>
<td>0.27</td>
<td>4.16</td>
</tr>
<tr>
<td>Cladophora</td>
<td>12.93</td>
<td>14.50</td>
<td>1.22</td>
<td>59.83</td>
<td>4.93</td>
<td>0.28</td>
<td>1.99</td>
</tr>
<tr>
<td>Duck weed</td>
<td>15.53</td>
<td>16.64</td>
<td>3.14</td>
<td>18.43</td>
<td>3.03</td>
<td>0.39</td>
<td>4.19</td>
</tr>
<tr>
<td>Milfoil</td>
<td>5.61</td>
<td>17.04</td>
<td>0.94</td>
<td>62.53</td>
<td>16.72</td>
<td>0.10</td>
<td>1.82</td>
</tr>
<tr>
<td>Pond weed</td>
<td>10.54</td>
<td>20.00</td>
<td>2.90</td>
<td>40.44</td>
<td>11.90</td>
<td>0.19</td>
<td>2.78</td>
</tr>
<tr>
<td>Vallisneria</td>
<td>14.57</td>
<td>20.74</td>
<td>2.50</td>
<td>37.90</td>
<td>5.82</td>
<td>0.34</td>
<td>3.09</td>
</tr>
</tbody>
</table>
Muztar *et al.* (1976) revealed that the water weeds had extremely variable total ash (18.43-62.53%), crude protein (5.61 to 15.5%) and contained relatively less crude fibre and ether extract than dehydrated alfalfa meal. The calcium content varied from 3.0 to 16.7 per cent as compared to 1.9 per cent for dehydrated alfalfa.

The inclusion of aquatic plants at low levels in poultry diets had shown better performance especially when they supply part of the total protein, they serve as a source of pigment for egg yolk and broiler skin (Maurice *et al.*, 1984).

Aquatic plant species accumulate secondary plant compounds and therefore offer greater potential than any other types of leaf protein sources for monogastrics. The high xanthophyll content in aquatic plants makes them superior to alfalfa meal in imparting colour to egg yolk and poultry skin pigmentation (Becerra *et al.*, 1995).

Among different aquatic plants, the *in-vitro* dry matter digestibility was highest for Azolla (52.2%) followed by that for duck weed (51.2), spirodella (40.0), water spinach (40.0), water lettuce (42.1) and the lowest in water hyacinth (33.3) (Ly *et al.*, 2002). They also reported that the *in-vitro* pepsin/pancreatin digestibility of N was highest for Azolla (76.6%) followed by duck weed (75.4), water lettuce (62.2), water spinach (54.6), spirodella (54.2) and water hyacinth (50.5%). Of these species, the cosmopolitan fern Azolla, is perhaps the most promising from the point of view of ease of cultivation, productivity and nutritive value.
2.2 Azolla

Azolla (mosquito fern, duckweed fern, fairy mass and water fern) is a floating fern in shallow water. It floats on the surface of water by means of numerous, small, closely overlapping scale like leaves, with their roots hanging in the water. They are extremely reduced in form and specialized, looking nothing like conventional fern but more resembling duckweed or some mosses. Azolla form a symbiotic relationship with the blue green algae, *Anabaena azollae* which fixes atmospheric nitrogen and convert to plant nitrogen. This had led to the plant being dubbed a “super plant”, as it can readily colonize areas of fresh water, and grow at great speed doubling its biomass every two to three days.

2.2.1 Morphology

An Azolla plant floating on the surface of the water is roughly triangular or circular in shape and rarely exceeds 3-4 cms (except in the species of *A. nilotica*). The stems are covered and hidden by small, alternate, imbricate leaves. Adventitious roots are formed on the lower part of the stem and grow vertically in the water. Each leaf is bi-lobed, the lower, achlorophyllous lobe ensuring floatation and the upper, chlorophyllous one developing a cavity that remains in contact with the external environment through structurally sophisticated pore. The cyanobacterium *Anaebaena azollae* occurs as filaments located on the plant stem apexes and inside the leaf cavities (Van Hove and Lejeune, 2002).
2.2.2 Taxonomy and biogeography

Azolla is the only genus in the family Azollaceae and has a worldwide distribution from temperate to tropical climates. There are seven species of Azolla, which are grouped in two sections. The Rhizosperma section comprises Azolla nilotica, native to east Africa, and A. pinnata, of which two varieties are generally recognized: A. pinnata var. pinnata, present throughout Africa, Madagaskar and in Australia, and A. pinnata var. imbricata from subtropical and tropical Asia. The Azolla section comprises: A. caroliniana, A. mexicana and A. microphylla from temperate, subtropical and tropical America respectively and A. falculoides and A. rubra from America and the Far East respectively (Van Hove and Lejeune, 2002).

2.2.3 Symbiotic Interaction with Anabaena azollae

Azolla hosts symbiotic blue green algae, Anabaena azollae, which is responsible for the fixation and assimilation of atmospheric nitrogen. Azolla, in turn, provides the carbon source and favorable environment for the growth and development of the algae. It is this unique symbiotic relationship that makes Azolla, a wonderful plant with high protein content (Kamalasanana Pillai et al., 2005).

2.2.4 Azolla in agriculture

Azolla can be used as a food, mosquito inhibitor, green manure, herbicide, water saver, water purifier, nitrogen fertilizer saver, drug and for reclaiming saline soils (Van Hove and Lejeune, 1996).
It has long been used by farmers, mainly in Asia, as feed for their animals and as green manure. A number of laboratory and field studies have shown an unquestionable beneficial effect of Azolla as an organic nitrogen fertilizer, mainly in terms of increasing rice grain yield. In addition, the presence of an Azolla mat on the surface of the water body has been shown to significantly reduce weed development, limit evapotranspiration, reduce volatilization of applied N fertilizers and purify water (Van Hove and Lejeune, 2002).

Azolla has long been used as a green manure and as a feed for poultry (Basak et al., 2002), pig (Becerra et al., 1990) and fish (Nwanna and Falaye, 1997).

Fresh azolla is used in the preparation of compost. Since the fern has an excellent carbon nitrogen ratio, it decomposes rapidly and accelerates the decomposition of other organic residues inside the compost pit and used as a biofertilizer in coffee plantations (Anand Titus and Geeta Pereira, 2007).

2.2.5 Yield

The plant multiplies rapidly and gives high dry matter yield in spite of its high water content. Singh (1982) reported that under conditions in Cuttack region of east coast, an annual production of fresh material was 347 tonnes/h in field and 321 tonnes/h in concrete tank.

Abeyratne (1982) reported a dry matter yield of 28 tonnes/h/year and concluded that the same is higher than that could be obtained from same area planted with grass, fodder or legume under Sri Lankan conditions.
Kamalasanana Pillai et al. (2005) reported that 500-600g of fresh Azolla can be harvested daily from a pit of 2 x 2 x 0.2 m after 10-15 days of culture.

2.3 Chemical composition of Azolla

There is a variation in the nutrient composition of Azolla meal in different studies which could be attributed to differences in the response of Azolla strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their growth morphology and composition. Moreover, species difference of Azolla could alter their composition. Furthermore, contamination with epiphytic algae could also be important to such a degree as to affect the results of chemical composition (Sanginga and Van Hove, 1989). Variations in the chemical composition of *Azolla pinnata* (Table 2.2) has been reported by authors Singh and Subudhi (1978), Tamang and Samanta (1991), Anand Titus and Geeta Pereira (2007), Ali and Leeson (1994), Khatun (1996), Parthasarathy et al. (2002), Alalade and Iyayi (2006) and Balaji et al. (2009).

Seyed Mozafar et al. (1990) reported that *Azolla microphylla* contained crude protein 25.33, ether extract 3.01, crude fibre 11.06 and total ash 23.59 per cent. The mineral content of Azolla was Ca 1.70, P 1.05 and AIA 6.81 per cent. The NDF and ADF content were 40.36 and 25.24%, respectively. The essential amino acid profile of azolla was favourably comparable to that of other vegetable proteins. However, lysine and sulphur containing amino acids, methionine and cystene were deficient in *Azolla microphylla* as is true for most of the leaf proteins. But it is rich source of arginine, histidine, isoleucine, serine and tryptophan.
Table 2.2: Chemical Composition (on % dry matter basis) of *Azolla pinnata*  

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<td><strong>Proximate Principles</strong></td>
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</tr>
<tr>
<td>Dry matter</td>
<td>90.12</td>
<td>90.59</td>
<td>90.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>89.70</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>9.1</td>
<td>14.13</td>
<td>-</td>
<td>12.50</td>
<td>12.38</td>
<td>13.60</td>
<td>12.70</td>
<td>14.90</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3-3.6</td>
<td>2.73</td>
<td>3.0-3.5</td>
<td>1.60</td>
<td>-</td>
<td>2.37</td>
<td>2.70</td>
<td>3.70</td>
</tr>
<tr>
<td>Total ash</td>
<td>10.50</td>
<td>20.35</td>
<td>10.00</td>
<td>36.10</td>
<td>-</td>
<td>12.30</td>
<td>16.20</td>
<td>17.00</td>
</tr>
<tr>
<td>NFE</td>
<td>-</td>
<td>47.42</td>
<td>-</td>
<td>45.71</td>
<td>-</td>
<td>36.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cell wall fractions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>-</td>
<td>67.54</td>
<td>-</td>
<td>47.80</td>
<td>44.57</td>
<td>-</td>
<td>47.08</td>
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<tr>
<td>Acid detergent fibre</td>
<td>-</td>
<td>51.96</td>
<td>-</td>
<td>46.70</td>
<td>33.41</td>
<td>-</td>
<td>12.76</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>-</td>
<td>15.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.76</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Lignin</td>
<td>-</td>
<td>17.48</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28.24</td>
<td>-</td>
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<tr>
<td><strong>Minerals</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Calcium</td>
<td>0.4-1.0</td>
<td>1.54</td>
<td>0.45-1.25</td>
<td>1.43</td>
<td>-</td>
<td>1.24</td>
<td>1.16</td>
<td>2.14</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.5-0.9</td>
<td>0.35</td>
<td>0.15-11.00</td>
<td>0.31</td>
<td>-</td>
<td>0.72</td>
<td>1.29</td>
<td>0.44</td>
</tr>
<tr>
<td>Potassium</td>
<td>2-4.5</td>
<td>-</td>
<td>0.25-5.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.25</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.5-0.65</td>
<td>-</td>
<td>0.25-5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td>Sulfur</td>
<td>-</td>
<td>-</td>
<td>0.20-0.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium</td>
<td>-</td>
<td>-</td>
<td>150-1250</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.79</td>
<td>-</td>
</tr>
<tr>
<td>Manganese, ppm</td>
<td>-</td>
<td>-</td>
<td>60-2500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>174.42</td>
<td>-</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>-</td>
<td>-</td>
<td>25-750</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>87.59</td>
<td>-</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>-</td>
<td>-</td>
<td>2-250</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.74</td>
<td>-</td>
</tr>
<tr>
<td>Iron, ppm</td>
<td>60-260</td>
<td>-</td>
<td>40-500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>755.73</td>
<td>-</td>
</tr>
<tr>
<td>Silica, ppm</td>
<td>-</td>
<td>15.98</td>
<td>0.15-3.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.82</td>
<td>-</td>
</tr>
</tbody>
</table>
Ali and Leeson (1995) and Alalade and Iyayi (2006) revealed that Azolla meal is rich in leucine, lysine, arginine and valine, while tryptophan and sulphur containing amino acid were deficient. The amino acid profile of Azolla reported by different Authors is presented in Table 2.3.

Becerra et al. (1995) analyzed the chemical composition of *Azolla microphylla* to contain 26.7 per cent crude protein, 15.1 per cent ash, 94.4 per cent moisture, 0.4 per cent phosphorus, 0.8 per cent calcium and 4.6 per cent ether extract and 11.2 per cent crude fibre on dry matter basis.

Parthasarathy et al. (2003) reported that the apparent and true metabolizable energy values of *A. pinnata* were 1529 and 1855 kcal/kg DM, respectively suggesting that composition of azolla has lower energy content when compared to bran and oil cakes such as sunflower cake.

Kamalasanana Pillai et al. (2005) reported that Azolla is very rich in proteins, essential amino acids, vitamins (vitamin A, vitamin B<sub>12</sub> and Beta-Carotene), growth promoter intermediaries and minerals like calcium, phosphorous, potassium, iron, copper, magnesium etc.

Alalade and Iyayi (2006) reported the gross energy content of *Azolla pinnata* as 2039 Kcal/kg DM while Balaji et al. (2009) recorded the gross energy of Azolla as 1807 kcal/kg DM.
Table 2.3: Amino acid composition of *Azolla pinnata*

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% DM</td>
<td>g/100g CP</td>
<td>% DM</td>
<td>g/100g CP</td>
<td>Chemical score (%)</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.62</td>
<td>3.80</td>
<td>0.98</td>
<td>4.58</td>
<td>130.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.25</td>
<td>1.50</td>
<td>0.34</td>
<td>1.59</td>
<td>45.4</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.15</td>
<td>0.90</td>
<td>0.18</td>
<td>0.84</td>
<td>24.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.66</td>
<td>4.00</td>
<td>0.87</td>
<td>4.07</td>
<td>116.3</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.08</td>
<td>0.50</td>
<td>0.39</td>
<td>1.82</td>
<td>52.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.82</td>
<td>5.00</td>
<td>1.15</td>
<td>5.37</td>
<td>153.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.69</td>
<td>4.20</td>
<td>0.93</td>
<td>4.35</td>
<td>124.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.28</td>
<td>7.70</td>
<td>1.65</td>
<td>7.71</td>
<td>220.3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.77</td>
<td>4.60</td>
<td>1.01</td>
<td>4.72</td>
<td>134.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.49</td>
<td>4.00</td>
<td>0.68</td>
<td>3.18</td>
<td>90.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.86</td>
<td>5.20</td>
<td>1.00</td>
<td>4.60</td>
<td>131.4</td>
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<td>Serine</td>
<td>0.66</td>
<td>4.00</td>
<td>0.90</td>
<td>4.21</td>
<td>120.3</td>
</tr>
<tr>
<td>Valine</td>
<td>0.84</td>
<td>5.10</td>
<td>1.18</td>
<td>5.51</td>
<td>157.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.95</td>
<td>5.80</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.26</td>
<td>1.60</td>
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<tr>
<td>Proline</td>
<td>0.67</td>
<td>4.00</td>
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<tr>
<td>Aspartic acid</td>
<td>1.37</td>
<td>8.30</td>
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<td>-</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.56</td>
<td>9.60</td>
<td>-</td>
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</tr>
</tbody>
</table>
2.4 Azolla as a feed ingredient in poultry and livestock

Singh et al. (1983) conducted growth cum digestibility trial on crossbred heifers and concluded that the sun-dried Azolla could replace concentrate mixture in the diet to the tune of 100 per cent.

Nik-Khah and Motaghi-Talab (1992) reported that the Azolla can be incorporated in the concentrate mixture of lactating cows at the level of up to 35 per cent. They also indicated that the differences between milk yields and milk constituents (fat, protein, ash, lactose and total solids) were not statistically significant (P>0.05).

Gavina (1993) revealed that there was no significant difference in the average final weight, feed consumption and feed conversion efficiency of pigs fed diets containing Azolla up to 40 per cent.

Sreemannaryana et al. (1993) incorporated 10, 15 and 20 per cent of Azolla in commercial feed mixture of New Zealand and Russian Grey Giant rabbits and observed highest average daily weight gain of 27.3 g in 20 per cent inclusion level compared to control, 10 and 15 per cent levels.

Tamang and Samanta (1993) indicated that the sun-dried azolla can be incorporated up to 20 per cent of concentrate mixture of goat kids without any deleterious effects on the performance, digestibility of various nutrients, carcass characteristics, haematological and biochemical parameters.
Duran (1994) reported that the aquatic plant *Azolla filiculoides* can replace up to 20 per cent of the soya bean protein with no deterioration in performance of growing and finishing pigs.

Azolla is one of the most nutritive aquatic plants, owing to its high crude protein and carotenoid contents and of generally good amino acid profile. It can be incorporated into the feed of fish (Nwanna and Falaye, 1997), pigs (Becerra *et al.*, 1990), broiler chickens (Basak *et al.*, 2002), ducks (Becerra *et al.*, 1995), rabbits (Sreemannaryana *et al.*, 1993), small ruminants (Tamang and Samanta, 1993) and ruminants (Nik-Khah and Motaghi-Talab, 1992).

Studies conducted with desi pigs fed with isonitrogenous concentrate mixtures containing sun-dried azolla revealed that Azolla can be incorporated up to 30 per cent without any considerable adverse effect on growth (Parthasarthy *et al.*, 2003).

### 2.5 Performance of broilers fed Azolla meal based diets

#### 2.5.1 Body weight gain

Basak *et al.* (2002) observed significant (P<0.01) improvement in live weight of broiler chicks than that of control diet fed birds (1579g) when they were fed with 5 per cent Azolla meal replacing sesame meal (1637g) at 6 weeks of age, while lower live weights were recorded in 10 and 15 per cent Azolla incorporated diets.

Parthasarathy *et al.* (2002) reported no significant difference in body weight gain of broilers on basal and 5 per cent Azolla diets whereas higher levels of inclusion resulted in significant (P<0.01) reduction in body weight gain (1752, 1676, 1650 g for 10, 15 and
20 per cent Azolla based diets, respectively) thereby concluded that the Azolla could safely be included at 5 per cent level, replacing 2.6 per cent wheat bran and 2.4 per cent fish meal without any adverse effect.

Recently, Balaji et al. (2009) reported that the cumulative body weight gain of broilers at sixth week of age in groups fed 0, 1.5, 3.0 and 4.5 per cent Azolla incorporated diets were statistically similar at 2123, 2123, 2066 and 2040g, respectively indicating that inclusion of Azolla up to 4.5 per cent in rations did not have any influence on body weight in broiler chicken.

Similarly, Dhumal et al. (2009) reported that the partial replacement of SBM at 2.5 and 5 per cent levels by Azolla meal has no adverse effect on weekly body weights of broilers. However, in third, fourth and fifth weeks, the body weights were higher than that of control.

2.5.2 Feed consumption

Castillo et al. (1981), Querubin et al. (1986a), Sreemannarayana et al. (1993) and Bhuyan et al. (1998) reported that the inclusion of azolla in broiler diet did not affect feed consumption up to 15 per cent level of inclusion. However, Bested and Morento (1985) stated that Azolla affected the palatability of the feed and reduced the feed consumption.

Basak et al. (2002) reported that feed consumption was almost similar in different treatment diets containing 0, 5, 10 and 15 per cent Azolla meal and the differences were non significant at all ages of the experimental period.
Parthsarathy et al. (2002) recorded that the feed consumption of broiler birds fed 0, 5, 10, 15 and 20 per cent Azolla diets to be 3925, 3924, 4034, 4018 and 4022g at 8 weeks of age and observed no significant difference among the groups.

Balaji et al. (2009) reported that the cumulative feed intake of birds up to sixth week of age was 3677, 3655, 3659 and 3722g for 0, 1.5, 3.0 and 4.5 per cent Azolla based diets, respectively revealing no significant difference among the dietary groups.

Dhumal et al. (2009) observed non-significant differences in weekly feed consumption between control, 2.5 and 5 per cent azolla fed groups.

2.5.3 Feed efficiency

Basak et al., (2002), observed significant difference in feed conversion ratio among the broilers fed with 0, 5, 10 and 15 per cent azolla meal during 5-6 weeks and 2-6 weeks periods. Poorest feed conversion ratio was obtained in treatment group which received 15 per cent azolla (2.5) that was similar to the treatment group which received 10 per cent azolla (2.38). Feed conversion ratios decreased significantly at 10 and 15 per cent Azolla meal in the diet. Similar results are also reported by Querubin et al. (1986b). Higher level of fibre and tannin in aquatic plant may be responsible for decreased nutrient utilization and ultimately decreased FCR (Muztar et al., 1978).

Parthasarathy et al. (2002) reported that the feed and protein efficiency ratios were similar in basal and 5 per cent Azolla diets whereas, these were significantly (P<0.01) decreased as level of Azolla increased from 10 to 20 per cent.
Balaji et al. (2009) reported that the mean cumulative feed efficiency of birds fed 0, 1.5, 3.0 and 4.5 per cent Azolla incorporated diets were 1.68, 1.71, 1.75 and 1.74, respectively revealing no significant difference among treatment groups.

Dhumal et al. (2009) reported no significant difference in feed conversion ratio among the control, 2.5 and 5 per cent Azolla dietary treatment groups, thus inferring that 5 per cent level of incorporation of Azolla in broiler diets may not adversely affect FCR.

2.5.4 Carcass characteristic and organametry

Basak et al. (2002) reported that the highest dressing percentage was observed in the birds fed with 5 per cent level of Azolla (72.16) when compared with 0, 10 and 15 per cent groups. However, they observed no significant differences in abdominal fat percentage within the different treatment groups. The mean giblet percentage was significantly higher in birds receiving 15 per cent Azolla (6.44) compared to other treatment groups.

Parthsarathy et al. (2002) reported significantly (P<0.01) higher dressing percentage (69.66%) at 8 weeks of age in broiler birds fed 5 per cent Azolla diet compared to control (67.79), 10 (65.76), 15 (65.38) and 20 per cent (65.19) Azolla fed groups. They also reported that there was a significant difference (P<0.01) in the weights of organs viz., heart, gizzard and giblets in birds fed 15 and 20 per cent Azolla diets.

Dhumal et al. (2009) reported that there were non-significant differences amongst the means of various traits such as carcass yield percentage, abdominal fat and abdominal pad thickness signifying the non-influence of Azolla meal on carcass quality.
2.5.5 Economics

Basak et al. (2002) reported that the Net profit per broiler was significantly highest in 5 per cent (Tk. 30.65) followed by control diet with sesame meal (Tk. 25.46) and lower in, 10 per cent (Tk. 19.86) and 15 per cent (Tk. 15.99) Azolla incorporated dietary treatments.

Dhumal et al. (2009) reported that the cost of feed per kg live weight gain of broilers fed 0, 2.5 and 5 per cent Azolla diets was Rs.30.01, 28.22 and 26.80, respectively. Hence, the use of Azolla is comparatively profitable when used in combination with Soyabean meal up to 5 per cent level of inclusion.

2.6 Performance of layers fed Azolla meal based diets

2.6.1 Egg production

Khatun (1996) reported that hen-day egg production of birds fed 5 per cent Azolla meal (digestible protein and digestible amino acid basis) based diet improved by 6.4 and 9.9 per cent over that of the control diet and diet with 10 per cent Azolla meal (total protein and total amino acid basis) respectively. Similarly, the hen day egg production of birds fed 10 per cent Azolla meal (digestible protein and digestible amino acid basis) improved by 2.0 and 5.7 per cent over the control diet and diet with 10 per cent Azolla meal (total protein and total amino acid basis). However, these effects were not statistically significant.

In another study, Khatun (1996) reported that hen-day egg production of birds fed 20 per cent Azolla on total protein and total amino acid was significantly lower than that
of hens fed control diet or diets with 15 or 20 per cent Azolla meal on digestible protein and digestible amino acid basis. These results indicated that Azolla meal can be used up to 15 per cent on a total protein and total amino acid basis for laying hen but egg production was reduced at 20 per cent level.

Khatun et al. (1999) found that Azolla meal could replace sesame meal on a digestible protein and digestible amino acid basis up to 200 g/kg of diet. The egg mass output and feed conversion ratio were significantly better but not when the diets were formulated on the total protein and total amino acid basis. He concluded that feeding azolla on total or digestible nutrient basis had no affect on egg quality except egg size and yolk color. Egg size improved when the diets were formulated on a digestible protein and digestible amino acid basis. Yolk color was significantly improved with increasing levels of azolla meal and longer period of feeding.

Alalade et al. (2007) reported that Azolla can be incorporated up to 15 per cent in diets of growing pullets without jeopardizing health and subsequent laying performance and egg characteristics except yolk weight.

2.6.2 Feed Consumption and Feed Efficiency

Alalade and Iyayi (2006) reported that average weekly feed intake was similar up to 5 per cent Azolla meal inclusion in diets while significantly reduced in 10 and 15 per cent Azolla meal. They also reported that feed efficiency improved from 3.13 on control diet to 2.54 in birds fed 10 per cent Azolla meal and to 2.55 on 15 per cent Azolla meal.
Alalade et al. (2007) reported that there were no significant difference in weekly feed intake among the groups fed 0 (731g), 10 (653g) and 15 per cent (659g) Azolla based diets. They also reported that the feed conversion ratio was 10.54, 9.33, 10.63 and 8.38 for the birds fed on 0, 5, 10 and 15 per cent Azolla meal in diets, respectively.

2.6.3 Body weight change

Singh and Subudhi (1978) reported that the White Leghorn birds receiving 5 per cent azolla with normal diet, grew faster than the control and the birds receiving 50 per cent feed with 16 per cent azolla had lower body weight than control, where as the birds receiving 75 per cent feed with 12.5 per cent were comparable to control. Thus they concluded that about 20 to 25 per cent of commercial feed can be replaced by the fresh azolla.

Alalade and Iyayi (2006) recorded an average weekly weight gain of 95.43, 95.22, 98.62 and 93.44g in egg-type chicks fed diets containing 0, 5, 10 and 15 per cent Azolla meal which were not significantly different from one another.

In an another study, Alalade et al. (2007) reported that the average body weight at 18 weeks were 1516, 1523, 1480 and 1435g for the groups fed diets containing Azolla meal at 0, 5, 10 and 15 per cent respectively. However, birds on 5 per cent azolla meal did non- statistically better than those on the control diet.

2.6.4 Egg quality

Alalade et al. (2007) reported that egg quality characteristics were similar among the birds fed Azolla incorporated diets during pullet stage (8-18 weeks). However, the
yolk weight decreased with the values of 12.94, 12.41, 12.18 and 12.73g for 0, 5, 10 and 15 per cent Azolla meal fed at grower phase, respectively.

2.6.5 Economics

Namra et al. (2010) obtained optimum economical efficiency and relative economical efficiency values for the control and treatment groups fed on restricted diet along with free fresh azolla at 45, 30 and 15 per cent levels. The control group recorded poorest value. With increase in the levels of restricted diet, the economical efficiency and relative economic efficiency values extremely increased linearly and the values were about 1.49, 1.84 and 3.02 times higher than the control group.

2.7 Effect of Azolla on the performance of other species of poultry

Cariaso (1992) studied the effect of feeding fresh azolla in Muscovy ducks and found that egg weight and albumen weight were higher in the birds fed 150g commercial pellet than those fed with 50g PSS (Palay-snail-shrimp) with and without Azolla, however egg yolk weight, shell weight and shell thickness were not affected by the dietary treatments. Egg yolk color was significantly darker from all the PSS with and without Azolla and Azolla at 15 and 30 percent further intensified the yellow color of egg. He also reported no statistical difference in egg production among the different treatments.

Shahjahan et al., (1992) reported that the body weight of Rhode Island Red chicks on day 49 of feeding were 3445, 3532, 3590, 4081 and 3272 g for the group fed control,
control with Spirodela, Lemna and Azolla mixture, or Azolla, Spirodela or Spirogyra diets, respectively.

Gavina (1993) conducted feeding study of duck supplemented with fresh azolla with 60 percent and 40 percent as the percentage of commercial feed in their diet. The results revealed that there was a significant difference in the average gain in the final weight of the duck, concluding that commercial feed could be reduced considerably in the ducks diet and thus encouraging azolla as feed supplement.

Becerra et al. (1995) studied the performance of duck by incorporating 15, 30, 45 and 60 per cent Azolla microphylla in boiled soya bean diet supplying 15.2, 17.9, 24.4 and 30.3 per cent of the total protein. The daily weight gain was found to decrease with increasing levels of Azolla when this exceeded 15 per cent of the dietary protein. Feed intake and FCR deteriorated with increasing level of Azolla (P<0.001).

In another trial, Becerra et al. (1995) noticed lower Azolla intake when they supplied 14.7, 17.2, 19.2 and 22.6 per cent of the total protein in diets with levels of 15, 25, 35 and 45 per cent respectively. The gains (g/day) were 32.6, 31.5, 30.2, 28.2 and 24.1 for the control, 15, 25, 35 and 45 per cent levels respectively, with increase in gain with azolla level beyond 15 per cent of the protein (P<0.001).

Lawas et al. (1998) noticed no significant difference in average feed efficiency (feed/egg) of laying ducks fed without (control) azolla (3.59) and ad lib azolla (3.70). Similarly no significant difference was noticed in average egg weight values for control (69.63g) and treatment group (69.80). They also reported that the egg production in
laying ducks fed 75 g of commercial feed and *ad lib* azolla was 59.3 per cent which was comparable with that of 60.2 in birds fed 150 g commercial feed/head/day.

### 2.8 Effect of Azolla on Livability and Nutrients Utilization

Buckingham *et al.* (1978) reported that the higher level of NDF in Azolla, affected the utilization of feed or feed efficiency in monogastic animals.

Alcantara and Querubin (1985) reported higher values of nitrogen digestibility in birds fed Azolla diets.

In goats, Tamang and Samanta (1993) reported that the digestibility coefficient of all nutrients except NFE, ether extract, cellulose and nitrogen, calcium and phosphorus balances were similar among the groups fed diets containing 0, 10 and 20 per cent sun-dried Azolla.

Ali and Leeson (1995) found that lower digestible protein and amino acids in Azolla than in rice polish and snail meal.

Khatun (1996) reported that no bird died in any treatment during experiments indicating that Azolla meal had no deleterious effect on livability.

Basak *et al.* (2002) reported that the survivability was cent per cent in all dietary treatment groups indicating that Azolla meal had no any deleterious effects on broilers.

Parthasarathy *et al.* (2002) observed that feeding of Azolla up to 20 per cent level did not have any adverse effect on livability of broiler birds. The per cent nitrogen retention of the broilers on 5 per cent (43.96) and 10 per cent Azolla diet (42.55) was
found to be comparable to that of control (42.71), however, significantly (P<0.01) lower in 15 per cent (37.62) and 20 per cent Azolla diet (36.08) at 8 week of age.

Dhumal et al. (2009) and Balaji et al. (2009) also observed that use of Azolla as a feed ingredient has no ill effect on the health of the birds. Azolla meal feeding in broilers improved the antibody titer values non-significantly as compared to control group at 35th day of age (Dhumal et al., 2009). Similar findings were observed by Quereshi et al. (1995) by exposing the chicken macrophages to water-soluble spirulina extract.

### 2.8.1 Protein efficiency

Khatun (1996) found lower digestibility of protein in *Azolla piñnata* at increasing level in the diet. Digestible protein level in Azolla is 56.6 per cent (Tamany et al., 1993) whereas digestible protein percentage of sesame meal is 89.8 per cent (NRC, 1994) which was why the control diet and diet containing 5 per cent Azolla meal might have shown better results.

Basak et al., (2002), reported that the best protein efficiency was observed in treatment with 5 per cent at all periods. On the other hand, it had 2.48 and 2.37 during 5-6 weeks and 2-6 weeks, which differed significantly from that of other treatments. Treatment groups with 10 per cent and 15 per cent had poorer protein efficiencies. Thus probably, due to the low digestibility, Azolla meal may be unfit as the sole source of feed for broilers (Buckingham et al., 1978). Poorer digestibility and higher fibre content of Azolla meal may be responsible for poorer protein efficiency at higher level of azolla inclusion.
2.8.2 Energy efficiency

Basak et al., (2002), reported that the Energy efficiency of broilers at different dietary treatments was highly significant during 5-6 and 2-6 weeks of age. At 5-6 weeks of age, the energy efficiency was best at the group which was receiving 5 per cent Azolla, which differed significantly from that of the groups which received 10 and 15 per cent Azolla but not from the control. At 2-6 weeks of age, energy efficiency was best at both 5 per cent and control and poorer energy efficiency in 10 and 15 per cent treatment groups. The protein efficiency also showed similar trend.

2.9 Fibre degrading enzymes

The use of alternative cereals and agro-industrial byproducts in poultry rations has a limitation because of presence of non-starch polysaccharides (NSPs) such as cellulose, arabinoxylans, pectins, beta glucans and low energy content. Poultry being monogastric, lacks fibre degrading enzymes and hence the use of exogenous enzymes facilitates the degradation of these polymers, thereby, making available the nutrients which would otherwise be excreted.

In layers, Sharma and Katoch (1993) observed a numerical increase in egg production when a fibre degrading enzyme was supplemented in a diet of 26 weeks old birds. Similar observations were also made by Jayanna and Devegowda (1993) and Mohandas and Devegowda (1993).

Choct et al. (1995) stated that suitable enzyme combination strategies from different feed ingredients might result in an increase in feed intake, stimulation of
growth, improvement of feed conversion and would overcome the problem of wet litter, all of which ultimately would culminate in the cost effectiveness of diets. Enzyme supplementation not only enhances bird performance and feed conversion, but also lessens the environmental problems (Yi et al., 1996).

Rajeshwara Rao and Devegowda (1996) reported that supplementation of enzyme to diets based on SFE and DORB improved the performance of broilers which was attributed to improved nutrient digestibility.

Zang Su min et al. (1996) reported that addition of 0.5 per cent compounded enzyme to the basal diets significantly (p<0.01) decreased the feed intake in layers. The improvement in the feed efficiency due to addition of single or compounded enzyme was also reported by Francesh et al. (1995).

However, Chennegowda et al. (2001) showed a marginal (non-significant) improvement in weight gain by 3.6 to 4.2 per cent in the groups fed 20 per cent sunflower extraction with commercial enzyme preparations (xylanase and pectinase or xylanase and cellulose) which was attributed to improved digestibility of NSPs by enzyme supplementation only at sufficient levels of substrate (20% SFE) as feed enzyme have higher Km.

Ponnuvel et al. (2001) observed that the per cent hen-day and hen-housed egg production, egg weight and feed efficiency were statistically comparable among groups fed standard layer diet and high fibre diets supplemented with cellulose at different levels (0.06, 0.12 and 0.18%). However, numerically better egg production and feed efficiency
were noticed when high fibre diet supplemented with cellulose. Further, they also noticed that the average daily feed intake was significantly lower in all the enzyme supplemented diets and standard layer diet fed groups when compared with unsupplemented high fibre ratio fed groups.

Exogenous enzymes in feed endows animals with additional metabolic arsenal to fight undigestible feed components as well as anti nutritional factors (ANF). Use of specific enzymes like xylanase, pectinase and cellulase could breakdown plant fibre releasing energy as well as increasing the protein digestibility due to better accessibility of protein when the fibre gets broken down (Saxena et al., 2006).

In general, it can be opined that azolla meal appears to be a valuable non-conventional feedstuff for broilers and it might prove still better than being observed in the above study, if energy and protein in the diets are optimally appropriated (since non-isonitrogenous and non-isocaloric diets used in several previous trials) beyond the level of 15 per cent that has been tested.