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AZOLLA AS AN ECONOMIC SUBSTITUTE TO SOYBEAN BASED FEED FOR POULTRY

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Abstract
A study on azolla as an economical substitute to soybean based poultry feed was conducted. The experiment consisted of four treatments laid out in Completely Randomized Design (CRD) with five replications. Each experimental unit consisted of three birds. For this purpose, eight-day old, unsexed Hubbard broiler chicks were weighed and randomly assigned to each treatment. Four different levels of azolla meal were incorporated in the starter and finisher ration at a level of 0%, 10%, 15%, and 20% respectively. Body weight, weight gain, feed consumption, and feed efficiency were measured. The average cumulative weekly body weight for all weeks were found to be significantly different (P<0.05) for the various treatments. Maximum cumulative body weight (1360 g) was found in broiler fed T2 ration in 5th week. Similar trend was also observed in 6th week showing highest cumulative weight (1882 g) in control diet (T1) which was at par with T2 (1841 g). Significantly highest (420.3 g) live weight gain was observed in T2 diet during 5th week. Overall mean of weekly feed consumption was recorded highest (784.33 g) in T2 diet. The economic analysis of net income/kg live weight was the highest (Rs 18.86) in T1 which was at par with T2 (Rs 18.35). Likewise, cost of production/bird was highest (Rs 124.5) in T1 and lowest (Rs 112.2) in T4. The results show that azolla meal could be utilized to substitute up to 10% soybean meal from overall production and economic point of view.

Keywords: Azolla; Poultry; Feed; Sustainable; Protein; Substitute; Economic

Introduction
Poultry farming has emerged as a major income generating enterprise in agriculture sector within the reach of the poor, women, marginal farmers, and entrepreneurs in Nepal. It has been playing a significant role in the rural economy and its contribution to national GDP and AGDP is estimated to be around 3% and 10% respectively involving more than 60,000 families (USAID, 2008).

Feed alone costs 65 to 75% of the total broiler production and ultimately results in high price of poultry meat (Johari and Hussain, 1996). Chicken has very limited ability to meet their protein needs from non-protein sources. Typical broiler ration will contain 22 to 24% protein. Protein synthesis in chickens is required at a very rapid rate for compensating the broken tissues of the adult body (Banerjee, 2000). The feeding of poultry is done through concentrates. Commercial feeds are expensive for average Nepalese farmers as more than 55 percent of Nepal’s population lives below the international poverty line of $1.25 per day (FTF, 2010). Therefore, the poultry rearing farmers are in search of economical and sustainable feed ingredients for poultry feeding. Many researchers are working on different materials to be used for economic broiler feed ration formulation (Olorunfemi et al., 2006; Fasuyi et al., 2007; Agbede, 2003; Abeke et. al., 2008). Azolla has shown a great potential to be included as a source of protein for duck ration (Bacerra et al., 1995) and egg type chicks (Alalade and Iyayi, 2006).

Azolla is a floating fern very rich in protein, essential amino acids, vitamins (Vit. A, Vit. B12, Beta carotene), growth promoter intermediaries and minerals like calcium, phosphorus, potassium, ferrous, copper, magnesium, etc. (Kamalasanana et al., 2002). A blue-green algae (Anabaena azollae) lives in the cavities of azolla leaves and fixes nitrogen from the atmosphere. Azolla has been used for different agricultural purposes (Akhter et al., 2002; Krishnakumar et al., 2005; Hossain et al., 2001; Tahmid et. al. 2002). Azolla has been used in animal feed ration as well (Sheeno and Sahu, 2006). The bio-composition of azolla makes it one of the most economic, efficient and sustainable feed substitute for poultry (Kamalasanana et al., 2002). Incorporation of azolla as an alternative protein ingredient in poultry ration could make poultry production economical.
Materials and Method

Experimental Site
The experiment was conducted at livestock farm of Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan.

Husbandry/Experimental Design
Sixty, day old Hubbard chicks were obtained from commercial hatchery and reared in battery brooder for one week. After brooding, chicks were transferred to experimental pen. The experiment was conducted in Completely Randomized Design (CRD) consisting of four treatments along with five replications. Each experimental unit consisted of 3 birds.

Azolla Meal Preparation
Whole plants of Azolla pinnata were harvested in sufficient quantities from Rupa Lake, Pokhara; Sangle river, Kathmandu and IAAS fish pond. The collected azolla was sun dried for three days until they became crispy while retaining greenish coloration. The dried leaves were then milled and used as feed ingredient. Chemical composition of the azolla was analyzed and is presented in Table 1. The analysis was carried out according to the standard methods of A.O.A.C. (1990).

Table 1: Chemical composition of Azolla

| Constituents       | Percentage |
|--------------------|------------|
| Dry matter         | 89.1       |
| Crude protein      | 24.18      |
| Crude fiber        | 15.41      |
| Ether extract      | 3.35       |
| Nitrogen free extract | 30.04  |
| Total ash          | 16.12      |

Ration Formulation
Two types of rations, one ration for broiler starter (0-4 weeks) and another ration for broiler finisher (4-6 weeks) were formulated (Table 2 and Table 3).

Feeding Trial
4 treatments with varying levels of Azolla meal were prepared as follows:

Table 2: Details of treatment composition (ingredients) and nutrient composition used in broiler starter rations (0-4 weeks)

| Ingredients          | Treatments |
|----------------------|------------|
|                      | T1  | T2  | T3  | T4  |
| Maize                | 582 | 544 | 526 | 529 |
| Rice polish          | 50  | 60  | 53  | 22  |
| Azolla meal           | 0   | 100 | 150 | 200 |
| Soybean meal         | 310 | 256 | 232 | 216 |
| Bone meal            | 20  | 20  | 20  | 20  |
| Marble               | 30  | 15  | 14  | 10  |
| Common salt          | 2   | 2   | 2   | 2   |
| Vit.–Min. Premix     | +   | +   | +   | +   |

Calculated nutrient composition
ME Kcal/kg 2886.8 2840.3 2802.75 2775.8
CP% 20.788 20.276 20.020 20.025
CF% 3.748 4.658 5.039 5.327
Ca% 1.721 1.284 1.298 1.205
Av. P% 0.671 0.766 0.800 0.815

Table 3: Details of treatment composition (ingredients) and nutrient composition used in broiler finisher rations (5-6 weeks)

| Ingredients          | Treatments |
|----------------------|------------|
|                      | T1  | T2  | T3  | T4  |
| Maize                | 625 | 646 | 620 | 598 |
| Rice polish          | 100 | 22  | 20  | 10  |
| Azolla meal           | 0   | 100 | 150 | 200 |
| Soybean meal         | 250 | 220 | 199 | 181 |
| Bone meal            | 10  | 5   | 5   | 5   |
| Marble               | 10  | 5   | 4   | 4   |
| Common salt          | 2   | 2   | 2   | 2   |
| Vit.–Min. Premix     | +   | +   | +   | +   |

Calculated nutrient composition
ME Kcal/kg 3015.50 2990.33 2949.85 2905.73
CP% 18.149 18.036 18.074 18.019
CF% 2.897 3.228 3.753 4.196
Ca% 0.725 0.517 0.531 0.581
Av. P% 0.604 0.548 0.589 0.621
General Management
Available brooder was washed, cleaned, and disinfected using Kohrsolin @ 0.03% solution. Floors, interior of the walls and roof were scrubbed and left over litters were removed from the brooder house. The poultry house was thoroughly cleaned. Before placing the litter in poultry house, dust lime was applied as disinfectant. In all experimental pens, rice husk was used as bedding material. The thickness of litter on floor was made to 10 cm. The birds were fed ad libitum in all treatments up to the 6th week of age.

Data Collection
Weekly body weight and body weight gain
The initial and weekly body weights of the birds were taken. Body weight gain was obtained by subtracting previous weight of the birds from their corresponding body weight for each week.

Feed and Water Consumption
The chicks of different treatments were reared under similar management and environmental conditions. Feed was offered ad libitum every day during the entire experimental period. Weekly ration consumption of each treatment was recorded by subtracting the weight of the left over ration from the weight of ration offered in each week. Water pots were thoroughly cleaned before watering each time. Fresh water was made available ad libitum during the experimental period.

Feed Conversion Ratio
The weekly feed conversion ratio of birds in each treatment was determined by dividing the weekly feed intake by their respective weight gain.

Economics of Production
The economics of broiler production was calculated at the end of the experiment. Gross expenditure was calculated by adding the cost of chicks, feeds, medicines, management, and labor cost. The gross income was obtained by adding selling price of the birds (NRs 75/bird) [USD 1 = Rs. 75] and litter (Rs 30/bag) at local market. The different economic parameters were calculated as follows.

Net income per bird (Rs.) = [Gross income (Rs.) – Gross expenditure (Rs.)] / No. of live birds

Net income per kg live weight (Rs.) = Net income of birds (Rs.) / Total live weight of birds (kg)

Cost of production per bird (Rs.) = Gross expenditure (Rs.) / Total number of birds in treatment

Statistical Analysis
The data recorded on different parameters during experiment were first tabulated in Microsoft Excel and then Analysis of Variance (ANOVA) for all data was statistically analyzed using MSTAT-C computer software package.

Results and Discussion
Feed Consumption
The analysis of variance showed a non-significant difference (P>0.05) among the treatments during the second and third week of the experimental period (Table 4). A significant difference in the average weekly feed consumption was observed in the 4th week. The highest feed consumption (850.0 g) was found in T4 which was at par with T2 (836.0 g) and T1 (799.3 g). The lowest feed consumption was found in T3 (701.7 g). Likewise in the fifth week, significantly highest feed consumption (1031.0 g) was found in T2 which was at par with T3 (971.3 g) and the lowest feed consumption was found in T4 (856.0 g). In the 6th week, significantly highest feed consumption was found in T2 (1202.0 g) which was at par with T1 (1182.0 g) and T3 (1111.0 g). The lowest feed consumption was found in T4 (974.0 g).

Body Weight
A significant difference (P<0.05) between the treatments was observed in all weeks for body weight (Table 5). During the 6th week, significantly highest body weight was found in T1 (1882.0 g) which was at par with T2 (1841.0) and the lowest body weight was found in T4 (1517 g).

The results are similar to Cambel (1984) who found better result using 10% and 15% AZM. The higher level of azolla (T3 and T4) meal resulted in poor growth of the broilers as compared to the growth of the broilers in T1 and T2 treatments.

Weekly Weight Gain
Significantly highest weekly weight gain (137.7 g) in 2nd week was recorded in T1 and the lowest weight gain (117.7 g) was recorded in T4 which was at par with T2 (122.3 g) and T3 (119.0 g) (Table 6). In the 6th week, significantly highest weight gain was observed in T1 (525.3 g) and the lowest weight gain was observed in T4 (362.0 g).

A significant difference (P<0.05) in the FCR was observed in all weeks (Table 7). In the sixth week poorer FCR (2.690) was observed in T4 which was at par with T3 (2.620) and superior FCR (2.252) was observed in T1.
Table 4: Average weekly feed consumption (g) of Hubbard broilers fed with diets using azolla meal as a feed ingredient in broiler ration

| Treatment  | Period (week) and feed consumption (g) | Overall mean |
|------------|--------------------------------------|--------------|
|            | 2  | 3  | 4  | 5  | 6  |                |
| T1 = AZM 0%| 290.7 | 577.9 | 799.3<sup>a</sup> | 856.0<sup>b</sup> | 1182.0<sup>a</sup> | 741.18       |
| T2 = AZM 10%| 285.3 | 569.3 | 836.0<sup>a</sup> | 1031.0<sup>a</sup> | 1202.0<sup>a</sup> | 784.72       |
| T3 = AZM 15%| 291.0 | 567.7 | 701.7<sup>b</sup> | 971.3<sup>a</sup> | 1111.0<sup>a</sup> | 728.54       |
| T4 = AZM 20%| 295.3 | 629.0 | 850.0<sup>a</sup> | 834.3<sup>b</sup> | 974.0<sup>b</sup> | 716.52       |
| Mean       | 290.6 | 586.0 | 779.2 | 921.3 | 1139.7 |               |
| Probability| 0.942 | 0.1898 | 0.0104 | 0.0000 | 0.0027 |               |
| LSD        | 34.38 | 65.07 | 87.64 | 65.01 | 114.5  |               |
| SE<sub>m</sub> | 11.47 | 21.70 | 29.23 | 21.68 | 38.21  |               |
| CV%        | 8.83  | 8.28  | 8.20  | 5.25  | 7.65   |               |

Means within the column followed by different superscripts are significantly different (P<0.05) by LSD.

Table 5: Average cumulative weight (g) of Hubbard broilers fed with diets using azolla meal as a feed ingredient in broiler ration

| Treatment  | Period (week) and feed consumption (g) |                |
|------------|--------------------------------------|--------------|
|            | Initial wt. | 2  | 3  | 4  | 5  | 6  |                |
| T1 = AZM 0%| 159.0<sup>a</sup> | 296.7<sup>a</sup> | 587.7<sup>a</sup> | 973.3<sup>a</sup> | 1356.7<sup>a</sup> | 1882.0<sup>a</sup> | 1722.17       |
| T2 = AZM 10%| 150.3<sup>b</sup> | 272.7<sup>b</sup> | 545.3<sup>b</sup> | 940.0<sup>a</sup> | 1360.3<sup>a</sup> | 1840.7<sup>a</sup>       |
| T3 = AZM 15%| 148.7<sup>b</sup> | 267.7<sup>b</sup> | 520.0<sup>b</sup> | 837.7<sup>b</sup> | 1224.7<sup>b</sup> | 1648.7<sup>b</sup>       |
| T4 = AZM 20%| 146.0<sup>b</sup> | 263.7<sup>b</sup> | 516.0<sup>b</sup> | 840.3<sup>b</sup> | 1224.7<sup>b</sup> | 1648.7<sup>b</sup>       |
| Mean       | 151.0  | 275.2 | 542.25 | 897.8 | 1274.25 | 1727.21       |
| Probability| 0.0011 | 0.0029 | 0.0003 | 0.0000 | 0.0000  | 0.0000         |
| LSD        | 5.699  | 16.60 | 29.42 | 43.51 | 55.91   | 75.37          |
| SE<sub>m</sub> | 1.901  | 5.538 | 9.813  | 14.51 | 18.65   | 25.14          |
| CV%        | 2.82   | 4.50  | 4.05  | 3.61  | 3.27    | 3.26           |

Means within the column followed by different superscripts are significantly different (P<0.05) by LSD.

Table 6: Average weekly body weight gain (g) of Hubbard broilers fed with diets using azolla meal as a feed ingredient in broiler ration

| Treatment  | Period (week) and feed consumption (g) |                |
|------------|--------------------------------------|--------------|
|            | 2  | 3  | 4  | 5  | 6  |                |
| T1 = AZM 0%| 137.7<sup>a</sup> | 321.0<sup>a</sup> | 385.7<sup>a</sup> | 383.3<sup>b</sup> | 525.3<sup>a</sup> | 344.6       |
| T2 = AZM 10%| 122.3<sup>b</sup> | 272.7<sup>a</sup> | 394.7<sup>a</sup> | 420.3<sup>a</sup> | 480.3<sup>b</sup> | 338.1       |
| T3 = AZM 15%| 119.0<sup>b</sup> | 252.3<sup>b</sup> | 317.7<sup>b</sup> | 387.0<sup>b</sup> | 424.0<sup>c</sup> | 300.0       |
| T4 = AZM 20%| 117.7<sup>b</sup> | 252.3<sup>b</sup> | 324.3<sup>b</sup> | 315.0<sup>c</sup> | 362.0<sup>d</sup> | 274.3       |
| Mean       | 124.17 | 267.07 | 355.6 | 376.4 | 447.9  |               |
| Probability| 0.0306 | 0.0012 | 0.0000 | 0.0000 | 0.0000  |               |
| LSD        | 14.12  | 19.00 | 27.99 | 21.34 | 41.69   |               |
| SE<sub>m</sub> | 4.710  | 5.337 | 9.336  | 7.119 | 13.91   |               |
| CV%        | 8.48   | 5.31  | 5.87   | 4.23  | 6.94    |               |

Means within the column followed by different superscripts are significantly different (P<0.05) by LSD.
### Table 7: Average feed conversion ratio of Hubbard broilers fed with diets using azolla meal as a feed ingredient in broiler ration

| Treatment | Period (week) and feed consumption (g) | Overall mean |
|-----------|---------------------------------------|--------------|
|           | 2          | 3          | 4          | 5          | 6          |             |
| T₁ = AZM 0% | 2.110<sup>c</sup> | 1.984<sup>c</sup> | 2.072<sup>c</sup> | 2.236<sup>c</sup> | 2.252<sup>c</sup> | 2.13         |
| T₂ = AZM 10% | 2.336<sup>b</sup> | 2.090<sup>c</sup> | 2.114<sup>c</sup> | 2.456<sup>b</sup> | 2.5<sup>b</sup> | 2.30         |
| T₃ = AZM 15% | 2.446<sup>ab</sup> | 2.250<sup>b</sup> | 2.210<sup>b</sup> | 2.510<sup>b</sup> | 2.620<sup>a</sup> | 2.41         |
| T₄ = AZM 20% | 2.510<sup>a</sup> | 2.490<sup>a</sup> | 2.620<sup>a</sup> | 2.650<sup>a</sup> | 6.690<sup>a</sup> | 2.59         |
| Mean      | 2.35      | 2.20      | 2.26      | 2.46      | 2.52      |             |
| Probability | 0.0001 | 0.0000 | 0.0000 | 0.0001 | 0.0000 |             |
| LSD       | 0.1469   | 0.1406   | 0.1122   | 0.1406   | 0.09481  |             |
| SEm<sup>+</sup> | 0.04899 | 0.04690 | 0.03742 | 0.04690 | 0.03162 |             |
| CV%       | 4.62     | 4.84     | 3.72     | 4.29     | 2.77     |             |

Means within the column followed by different superscripts are significantly different (P<0.05) by LSD.

The above results are similar to Basak et al. (2002) who reported poorest feed conversion ratio in 15% AZM included diets. This might be due to higher fiber content of azolla. Higher level of fiber and tannin in aquatic plant may be responsible for decreasing the nutrient utilization and ultimately decrease FCR (Muzlar et al., 1978).

**Survivability**

None of the birds died in any treatments during the experimental period. This indicates that azolla meal had no deleterious effects on broilers. The results are similar with Basak et al. (2002) and Castillo et al. (1981) who also found no toxic effect of dietary azolla.

**Economics of Broiler Production**

The analysis of variance was statistically significant (P<0.05) for all the economic parameters (Table 8). The cost of production/bird was highest (Rs124.5) in T1 which was at par with T2 (Rs 122.6) and the lowest cost of production/bird (Rs 112.2) was recorded in T4 which was at par with T3 (Rs 115.3). Similarly, the cost of production/kg live weight ( Rs 66.14) was recorded in T1 which was at par with T2 (Rs 66.65). Likewise, highest gross income/bird was found in T1 (Rs 160.0) which was at par with T2 (Rs 156.5) and the lowest gross income/bird was found in T4 (Rs 129.0).

Net income/bird was observed highest in T1 (Rs 35.50) which was at par with T2 (Rs 33.88) and the lowest net income/bird was observed in T4 (Rs 16.80). Likewise, the net income/kg live weight was found highest (Rs 18.86) in T1 which was at par with T2 (Rs 18.35) and the lowest net income/kg live weight was in T4 (Rs 11.02). The percentage net income was recorded maximum (28.54%) in T1 which was at par with T2 (27.59%) and minimum on T4 (14.93%).

**Conclusion**

The use of azolla meal as a feed ingredient in broiler ration was investigated. The experiment consisted of four treatments of azolla meal at 0%, 10%, 15% and 20%, laid out in the completely randomized design (CRD) with five replications. Each experimental unit consisted of three birds. Two types of rations, broiler starter (0 to 4th week) and finisher (5th to 6th week) were fed. The highest average total feed consumption from 2nd to 6th weeks of age was obtained on T2 (3923.6 g) and the lowest on T4 (3582.6 g). The highest final average body weight was obtained on T1 (1882 g) which was at par with T2 (1841 g) and the lowest body weight was found on T4 (1517.7 g). The average weekly body weight gain of broilers observed was statistically significant (P<0.05) for all weeks except 2nd week. The average weekly feed conversion ratio was the best (2.13) in control (T1) diet and was at par with T2 (2.30) and poorest in T4 (2.59). Overall economic analysis of broiler production was observed to be significantly different (P<0.05) among the treatments.

The inclusion of azolla meal at 10% (T2) was either superior or at par with control diet (T1) with respect to overall performance and net economic returns. From the results it can be concluded that broilers could be raised successfully without compromising performance while substituting soybean meal up to 10% with azolla meal.
Table 8: Cost of production and net returns at different dietary treatments

| Treatment        | Gross expenditure (Rs) | Cost of production/bird (Rs) | Cost of production/kg live weight (Rs) | Gross income (Rs) | Gross income/bird (Rs) | Net income (Rs) | Net income/bird (Rs) | Net income/kg live weight (Rs) | Net income (%) | Probability | LSD | SEm | CV% |
|------------------|------------------------|-----------------------------|----------------------------------------|-------------------|------------------------|----------------|---------------------|--------------------------------|---------------|-------------|------|------|------|
| T1 = AZM 0%      | 373.4<sup>a</sup>      | 124.5<sup>a</sup>           | 66.14<sup>c</sup>                     | 479.9<sup>a</sup> | 160.0<sup>a</sup>      | 106.5<sup>a</sup> | 35.50<sup>a</sup>   | 18.86<sup>a</sup>                   | 28.54<sup>a</sup> | 0.0000      | 10.35| 3.453| 2.17 |
| T2 = AZM 10%     | 367.7<sup>a</sup>      | 122.6<sup>a</sup>           | 66.65<sup>c</sup>                     | 469.4<sup>a</sup> | 156.5<sup>a</sup>      | 101.6<sup>a</sup> | 33.88<sup>a</sup>   | 18.35<sup>a</sup>                   | 27.59<sup>a</sup> | 0.0000      |      |      |      |
| T3 = AZM 15%     | 345.9<sup>b</sup>      | 115.3<sup>b</sup>           | 69.93<sup>b</sup>                     | 420.4<sup>b</sup> | 140.1<sup>b</sup>      | 74.55<sup>b</sup> | 24.85<sup>b</sup>   | 15.07<sup>b</sup>                   | 21.56<sup>b</sup> | 0.0000      |      |      |      |
| T4 = AZM 20%     | 336.5<sup>b</sup>      | 112.2<sup>b</sup>           | 73.98<sup>a</sup>                     | 386.9<sup>c</sup> | 129.0<sup>c</sup>      | 50.39<sup>c</sup> | 16.80<sup>c</sup>   | 11.02<sup>c</sup>                   | 14.93<sup>c</sup> | 0.0000      |      |      |      |
| Mean             | 355.9                  | 118.6                        | 69.2                                   | 439.2             | 146.4                  | 83.3           | 27.8                | 15.8                            | 23.1           | 0.0000      | 10.35| 3.453| 2.17 |

Means within the column followed by different superscripts are significantly different (P<0.05) by LSD.
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