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Effect of dietary supplementation of *Azolla* on the nutrient utilization in the broiler *Japanese quail*

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Abstract
The study was conducted to investigate the effect of dietary supplementation of *Azolla* on the Nutrient utilization performance of broiler *Japanese quail*. A total of one hundred thirty-five day-old broiler *Japanese quail* chicks were randomly allocated to three treatment groups Based on ICAR (2013) nutrient requirement for poultry, feeding of quail was carried out in two phases i.e. growth phase I (1-3 weeks) and growth phase II (4-5 weeks) for all treatment groups and 3 types of diets were formulated, diet 1 as control (T_0) with no *Azolla*, diet 2 and 3 contained 5% (T_1) and 10% (T_2) *Azolla* replacing the ingredients in the control diet on DM basis. The duration of the experiment was 0-5 weeks in the poultry instructional and demonstration unit (PIDU) of the College of veterinary science and animal husbandry, Anjora, Durg in April 2022. The effect of dietary *Azolla* supplementation was studied on nutrient utilization. All the birds were fed ad libitum during the feeding trial, which lasted 35 days. During the end of the starter and finisher phase, two birds from each replicate were slaughtered. A faecal sample was collected to determine the nutrient digestibility of the Azolla on broiler *Japanese quail*. The nutrient composition of *Azolla* was DM -6.7%, CP -24%, crude fat -3.8%, crude fibre- 15%, NFE- 36.3%, Total ash- 16.6 and AIA- 3.64%. The result from this study showed, there was no significant effect of *Azolla* supplementation on CP metabolizability %. EE metabolizability was lowest in 5% *Azolla* group and was highest in the 10% *Azolla* group. The N, Ca and P retention was not altered significantly due to *Azolla* inclusion in the diet of quails.

Keywords: Dietary, *Azolla*, nutrient utilization, *Japanese quail*

1. Introduction
The demand for feed is rising as a result of the expanding human population. Foods with a plant origin cannot meet the increasing demands of people. Therefore, cattle and poultry play a critical role in filling the gap in this situation. The poultry industry plays a crucial role in ensuring food security and providing a low-cost source of animal protein.

The total population of poultry increased by 16.8% between the 19th and 20th livestock censuses (851.81 million birds) in India which indicates that there is an increasing need for poultry production. India ranks third globally in the production of eggs and eighth globally in the production of meat, according to Economic Survey 2021-2022.

The Indian Council of Medical Research recommends 11 kg of poultry meat per year, although the per-person availability is only 2.8 kg (ICMR, 2019). The cost of feed ingredients, which makes up about 60–70% of the overall cost of production and is the biggest barrier to the production of poultry due to the enormous supply-demand gap. A search for cheap unconventional feed ingredients in poultry feeding has been sparked by the world's diminishing feed resources, their rising costs, and the increased competition between humans and birds for food grains.

Quail, also known as "Bater" in Hindi, is a good source of dietary protein of the highest quality. Quail meat has less cholesterol, more carbohydrates, better crude protein, calcium, potassium, iron, copper, zinc, and vitamins A, B_1, B_2, and B_{12}.

Soybean meal is a common source of protein in chicken feed, establishing a different source of protein, such as *Azolla*, would be advantageous for cost-effective poultry rearing.

Azolla is a type of floating fern having a symbiotic association with nitrogen-fixing blue-green algae, which makes it a remarkable "super-plant" with a high protein content (Anitha *et al*., 2016) [2].

It can quickly colonize freshwater areas and grow extremely quickly, doubling its biomass in just 7–10 days. It is also simple to cultivate and produces more than legumes and grasses do.

Due to its great nutritional value and rapid development, it is known as a "green gold mine" and "super-plant" (Wagner, 1997) [15]. As a cattle feed, *Azolla* has enormous potential due to its high concentration of protein, necessary amino acids, vitamins (vitamin A, vitamin B_{12}, and β--carotene), growth boosters, and minerals (Henry *et al*., 2017) [7].

On a dry matter basis, *Azolla* meal had 24.50% crude protein, 3.70% ether extract, 14.90% crude fibre, 39.90% NFE, 17.00% total ash, 2.14% calcium, and 0.44% phosphorus. It has been suggested to feed *Azolla* (*Azolla pinnata)* to broiler and layer chickens (Basak *et al*., 2002) [4].

2. Material and Material

2.1 Location of research: The goal of the current study was to determine the effects of dietary levels of grounded dried *Azolla* the nutrient utilization in 135 healthy and abnormality-free day-old broiler *Japanese quail* for up to five weeks in the poultry instructional and demonstration unit (PIDU) of the College of Veterinary Science & A.H. Anjora, Durg, Chhattisgarh.

2.2 Design of experiment: Three treatment groups of three replicates and 15 birds in each had day-old chicks distributed randomly among them. Quail were fed in two phases, referred to as growth phases I and II, according to the ICAR (2013) recommendation. Three different types of Isocaloric and isonitrogenous diets were created. Diet 1 served as the control (T0) and contained only basal diet diets 2 and 3, on the other hand, contained 5% and 10% of Azolla on a DM basis along with a basal diet respectively.

Table 1: Experiment design

Particulars	Control group (T_0)	Treatment group (T_1)	Treatment group (T_2)
No. of broiler quails	45	45	45
Replicate	3	3	3
Quails/ replicate	15	15	15
Dried *Azolla* meal (%)	0	5	10

2.3 Cultivation, harvesting and storage of *Azolla*

Three steel tanks with dimensions of 4.0 x 3.5 feet (length x breadth) and a depth of 45 cm are used to cultivate *Azolla* at the goat unit of the College of veterinary science in A.H. Anjora, Durg, Chhattisgarh. Each tank is covered with a plastic sheet to prevent water leakage as well as a thin layer of fertile soil was subsequently evenly spread on that plastic sheet and water was added up to a height of 25-30 cm. After that, a small amount (2 Kg) of The Fresh *Azolla* was inoculated in that tank. *Azolla* rapidly multiplied and filled Following this, the grown *Azolla* was properly washed in clean water and harvested every week from the tanks, after that it shade dried for four to five days, making the dried *Azolla* brittle while preserving its green colour. *Azolla* has been collected, packaged, and kept airtight in plastic bags until utilized.

2.4 Metabolism trial

Three days of metabolism trial was conducted between the 24^{th} to 27^{th} days of experiments, to determine the nutrient metabolizability (DM, CP and EE) and nutrient balance (N, Ca and P) in Japanese quail. For this, the birds were kept in a metabolic cage in which there was provision for individual feeding, watering and the collection of excreta. During this period a weighed quantity of feed was offered to each bird daily and the left-over residue was weighed to calculate the total feed intake by the birds. The excreta voided over the same period were collected quantitatively. The composite droppings from each lot were dried at 100 °C to a constant weight and ground. The representative samples of test diet and excreta were analysed to know nutrient utilization and balance.

2.5 Proximate Analysis of Feed and Faeces

Analysis of feed and faeces by using the techniques in AOAC (2005) [3], for a number of proximate principles (moisture, crude protein, ether extract, and crude fibre) Inorganic phosphorus and calcium were also examined in the samples (Talapatra *et al*., 1940) [14]. (Fiske and Subbarao, 1925) [6].

2.6 Statistical Analysis

Snedecor and Cochran's Completely Randomized Design (CRD) was used to do a one-way Analysis of Variance (ANOVA) on the data in order to understand the findings (1994). Using Duncan's Multiple Range Test, the importance of the difference and interaction was evaluated (DMRT).

3. Result

Effect of different dietary inclusion levels of *Azolla* on nutrient utilization in *Japanese quail*

The effect of dietary inclusion of different levels of dried *Azolla* on intake (g/bird/day), outgo (g/bird/day), balance (g/bird/day) and metabolizability % of nutrients (DM, CP, and EE) in *Japanese quail* is presented in Table 2.

The data presented in the table revealed a non-significant ($p>0.05$) effect of different dietary levels on dry matter (g/bird/day) intake, outgo, balance and The DM intake during the metabolic trial in groups T_0, T_1 and T_2 was 23.74, 23.74 and 25.26 g respectively. The DM metabolizability % was 71.17, 69.47 and 63.27 in groups T_0, T_1 and T_2 respectively.

The effect of dietary inclusion of different levels of dried *Azolla* on crude protein intake recorded non-significant differences among the groups in the present study. The value of crude protein outgo by the birds in different groups did not vary significantly during the trial period. The per cent metabolizability of crude protein was 82.63%, 78.85% and 73.07% in groups T_0, T_1 and T_2, respectively which is also non-significant ($p>0.05$). (Table 2)

The values of ether extract (EE) intake due to different levels of dried *Azolla* recorded significant differences among the groups. The per cent retention of ether extract was significantly different among the groups and recorded at 93.24, 94.11 and 95.15% in groups T_0, T_1 and T_2 respectively. Alcantara and Querubip (1985) [1] reported that nutrient digestibility of crude protein, crude fat and crude fibre was not affected by the level (0, 5, 10, 15 and 20%) of *Azolla* in the ration of broiler chicken. Parthasarathy, (1995) [10] found that birds feeding of 5% *Azolla* did not show any significant difference in DM metabolizability from that of the control group and the values were 68% in the control and 69.02% in the 5% *Azolla* group. *Azolla* inclusion at 5% level exhibited the highest percentage of CP metabolizability (75.62%) than 0, 10, 15 and 20% dietary *Azolla*-supplemented birds. Our findings are supported by the findings of previous researchers Rana *et al*. (2017) [11] and Rathod *et al*. (2013) [12] were reported no significant ($p>0.05$) difference in DM, CP and EE

digestibility due to *Azolla* supplementation at 5% level in the diet as compared to the control group of broiler and *Japanese quail* respectively. Rathod *et al.* (2013) [12] reported no significant difference in DM metabolizability in *Japanese quail*.

Table 2: Effect of different dietary inclusion levels of *Azolla* on nutrient utilization in growing quails

Nutrient metabolizability %	Parameter	T_0 (Control)	T_1 (5% *Azolla*)	T_2 (10% *Azolla*)	Sig.	P value
Dry matter	Intake (g)	23.74±0.23	23.74±0.99	25.26±1.04	NS	0.394
	Outgo (g)	6.84±1.05	7.23±0.18	9.34±1.12	NS	0.187
	Balance (g)	16.89±1.08	16.51±0.93	15.92±0.33	NS	0.732
	Metabolizability (%)	71.17±4.50	69.47±1.22	63.27±3.17	NS	0.271
Crude protein	Intake (g)	5.41±0.051	5.37±0.22	5.59±0.23	NS	0.708
	Outgo (g)	0.93±0.17	1.13±0.05	1.51±0.18	NS	0.080
	Balance (g)	4.48±0.17	4.24±0.25	4.07±0.08	NS	0.350
	Metabolizability (%)	82.68±3.15	78.85±1.52	73.07±2.34	NS	0.080
Ether Extract	Intake (g)	0.96^{c}±0.00	1.12^{b}±0.05	1.43^{a}±0.05	S	**0.001
	Outgo (g)	0.06±0.01	0.07±0.00	0.07±0.03	NS	0.890
	Balance (g)	0.90^{c}±0.01	1.05^{b}±0.04	1.36^{a}±0.04	S	**0.000
	Metabolizability (%)	93.24^{ab}±1.16	94.11^{b}±0.55	95.15^{a}±1.37	S	*0.503

Superscripts are read row-wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different*($p<0.05$), **($p<0.01$), NS= Non-Significant

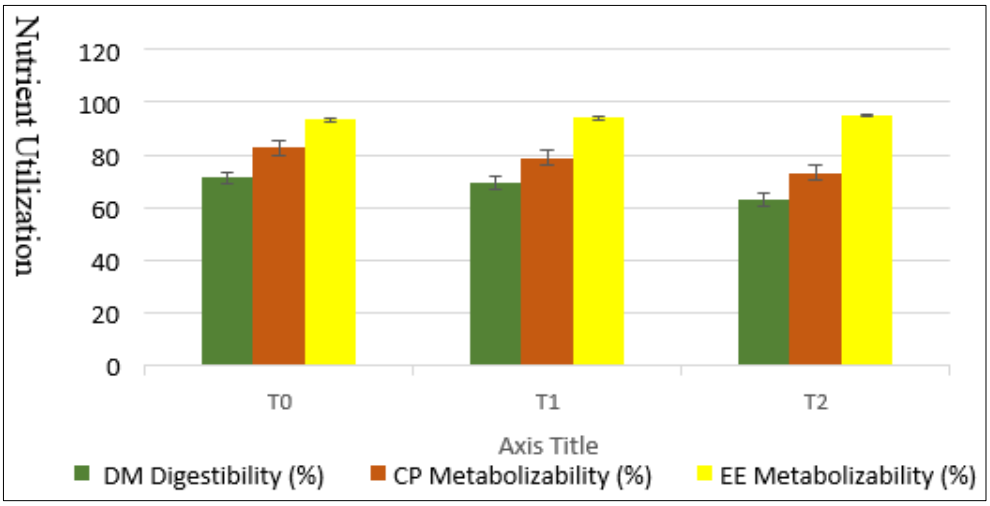

Fig 1: Effect of different dietary inclusion levels of *Azolla* on nutrient utilization in growing quails (0-35 d)

Nutrient Balance

The effect of *Azolla* inclusion on intake (g/ bird/ day), outgo (g/ bird/ day), balance (g/ bird/ day) and retention % of N, Ca and P in *Japanese quail* broilers.

In the current study, it was found that the impact of dietary inclusion of various quantities of dried *Azolla* on N intake, N outgo, and balance was statistically non-significant. In groups T_0, T_1, and T_2, the percentage of N retention was 82.68, 78.85, and 73.07%, respectively. The groups' intake, output, balance, and retention percentages of Ca and P as a result of varying dry *Azolla* inclusion levels did not statically differ ($p>0.05$). In different groups, the percentage of Ca retained ranged from 47.75 to 49.89% while the percentage of P retained ranged from 29.81 to 32.78%.

Similar to the current work, Rana *et al.* (2017) [11] found no impact of 5% dietary *Azolla* supplementation on the Ca and P retention in broilers.

Table 3: Effect of different dietary inclusion levels of *Azolla* on N, Ca and P retention in growing quails (0-35 d)

Retention (%)	Parameter	T_0 (control)	T_1 (5% *Azolla*)	T_2 (10% *Azolla*)	Sig.	P value
N	Intake (g)	0.87±0.00	0.86±0.03	0.89±0.03	NS	0.655
	Outgo (g)	0.15±0.02	0.17±0.00	0.24±0.03	NS	0.098
	Balance (g)	0.71±0.02	0.67±0.04	0.65±0.01	NS	0.361
	Retention (%)	82.68±3.15	78.85±1.52	73.07±2.34	NS	0.080
Ca	Intake (g)	0.23±0.01	0.27±0.003	0.24± 0.01	NS	0.140
	Outgo (g)	0.12±0.01	0.14±0.003	0.12±0.01	NS	0.152
	Balance (g)	0.11±0.00	0.13±0.01	0.12±0.003	NS	0.06
	Retention (%)	47.75±1.87	48.21±2.39	49.89±1.55	NS	0.036
P	Intake (g)	0.13±0.003	0.14±0.01	0.16±0.003	NS	0.052
	Outgo (g)	0.09±0.01	0.10±0.002	0.10±0.01	NS	0.67
	Balance (g)	0.04±0.01	0.04±0.00	0.05±0.00	NS	0.33
	Retention (%)	29.81±1.89	30.05±0.61	32.78±1.23	NS	0.17

Superscripts are read row-wise for comparison of means. NS= Non-Significant

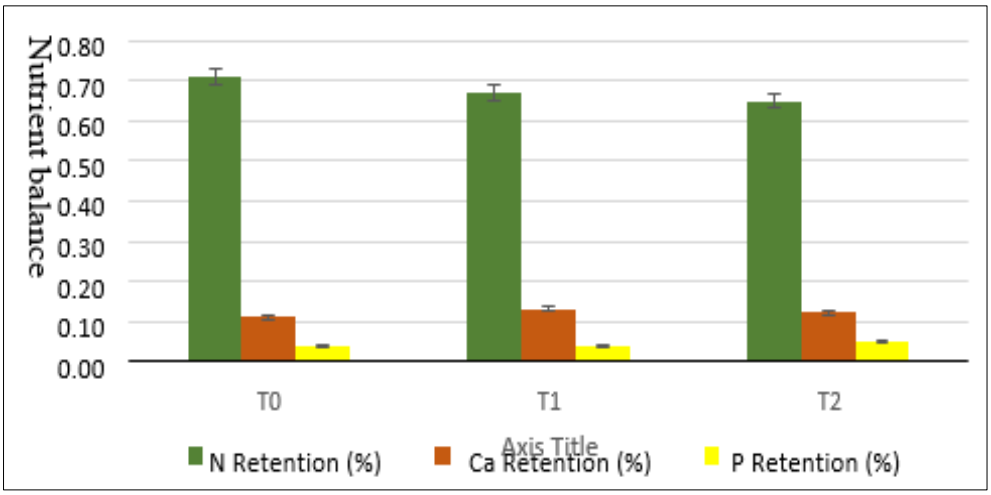

Fig 2: Effect of different dietary inclusion levels of *Azolla* on nutrient retention in growing quails (0-35 d)

4. Conclusion

The study revealed that the supplementation of shade-dried *Azolla* meal at A 5% level of *Azolla* inclusion, DM and CP metabolizability % were not affected significantly except for lowering EE metabolizability.

5. Acknowledgement

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