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Pawan Acharya
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Lokesh Gupta
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Surendra Singh
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Dashrath Singh Chundawat
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Hitesh Muwal
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Gaurav Singh
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Corresponding Author:
Pawan Acharya
Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

Effect of feeding *Azolla* leaf powder (*Azolla pinnata*) on the nutrients utilization of Kadaknath chicken (*Gallus domesticus*)

Pawan Acharya, Lokesh Gupta, Surendra Singh, Dashrath Singh Chundawat, Hitesh Muwal and Gaurav Singh

Abstract
The study was conducted to investigate the "Effect of feeding *Azolla* Leaf Powder (*Azolla pinnata*) on the performance of Kadaknath chicken (*Gallus domesticus*)." One hundred fifty Kadaknath chicks (four weeks old) were used in a completely randomized design in 5 treatments with 3 replications, each consisting of 10 chicks. The treatments were T_1: control, T_2: control group supplemented with *Azolla* leaf powder @ 2.5%, T_3: control group supplemented with *Azolla* leaf powder @ 5%, T_4: control group supplemented with *Azolla* leaf powder @ 7.5% and T_5: control group supplemented with *Azolla* leaf powder @ 10%. A metabolic trial was conducted at 16^{th} week of age with 3 days adaptation and 3 days collection period to study the digestibility of nutrients and balance of nitrogen. Significantly highest DM, CP, CF, EE, NFE intake and digestibility coefficient of DM and NFE was found in group of birds fed diets containing 5% ALP as compared to other treatment groups. Non-significant effect was found in digestibility coefficient of CP, CF and EE among different treatment groups.

Keywords: *Azolla* leaf powder, Kadaknath, nutrient utilization, digestibility

Introduction
Among the livestock occupations in India, poultry farming holds a unique place due to its huge potential to spur rapid economic growth at a low cost. It is one of agriculture's most lucrative industries since it provides wholesome meat and eggs for human consumption in the shortest amount of time. In India today, poultry is one of the agricultural sectors with the quickest growth rates. The majority of the protein and dietary needs are maintained by the chicken industry. The poultry industry in India has endured an exemplary transformation in structure and operation during the last two decades and modified into a mega-industry with the presence of a huge number of workers from a mere backward poultry farming that appears to be very fast. Feed costs are thought to be low in-home chicken production. The sustained availability of low-priced, high-quality feeds in India is critical if poultry production is to remain competitive and to continue to grow to meet the increasing consumer demand for eggs and meat (Ali 2007) [1]. The Salviniaceae family includes the watery free-floating fern known as *Azolla*. *Azolla*'s nutritional value has been extensively studied, and it is clear that it is a rich source of protein with nearly all of the essential amino acids needed for animal nutrition (particularly lysine). In addition, it offers macronutrients including calcium, magnesium, potassium, and vitamin A. These facts suggest that *Azolla* can be utilized as an unconventional feed with a protein supplement for a variety of animals, including ruminants, poultry, pigs, and fish (Pillai *et al*., 2002) [22]. Due to ease of cultivation, high productivity and good nutritive value, it is used as a beneficial fodder supplement by various researchers. *Azolla pinnata* has been tried as a feed for broiler chicken and was also used in diet formulations. *Azolla* contains (on DM basis) 20.4–28.5% crude protein, 2.37–6.70% ether extract, 12.6-17.5% crude fibre, 15.7–19.9% total ash, 30.0–47.0% nitrogen free extract, 48.2–54.8% neutral detergent fibre, 36.5–37.1% acid detergent fibre, 0.80–2.22% calcium and 0.35–1.39% total phosphorous (Swain *et al*. 2022) [37]. *Azolla* is incredibly rich in minerals including calcium, phosphorous, potassium, iron, copper, magnesium, and many others as well as proteins, vital amino acids,

vitamins (such vitamin A, B12, and -Carotene), growth promoter intermediates, and vitamins. The amount of fat and carbohydrates in *Azolla* is extremely little. According to the Natural Resources Development Project Vivekananda Kendra's assessment, its nutrient makeup makes it a very efficient and effective feed for animals. *Azolla* has a protein content that ranges from 25 to 35 percent of its dry matter and is readily assimilated by chickens. *Azolla*'s nutrient profile is essentially equal to that of conventional poultry feed, with the exception of its slightly lower calcium and higher protein content. *Azolla* is commonly used as biofertilizer as well as green manure in the paddy field. Now a days, *Azolla* (either fresh or in dried) is also used as a feed ingredient for ruminants and non-ruminants. Besides its utilization as biofertilizer and livestock feed, *Azolla*, the 'green gold mine' of the nature is also used as medicine, water purifier, human food and for production of biogas (Roy *et al*. 2016) [29]. The use of *Azolla* also increases organic matter and potassium contents of the soil (Bhuvaneshwari and Singh, 2015) [4].

Methodology

Preparation of *Azolla* meal

The *Azolla* culture was provided by the Poultry Farm, Department of Animal Production, Rajasthan College of Agriculture, MPUAT, Udaipur. A fresh *Azolla* culture that had been harvested and gathered after maturation was submerged in the tank's water. The harvested *Azolla* was washed and dried in a brine solution. The dried *Azolla* was pulverized with a grinder to a uniform size before being added to the feed.

Experimental bird details

The experiment was conducted at Poultry Farm, Department of Animal Production, Rajasthan College of Agriculture, MPUAT Udaipur. This research work was carried out with 150 Kadaknath chicks of four weeks of age procured from the Poultry Farm, Department of Animal Production, Rajasthan College of Agriculture, Udaipur. The chicks were wing banded and distributed randomly in five treatment groups, consisting of 30 chicks in each treatment group with three replications of 10 birds each.

Statistical analysis

The experiment was carried out using a completely randomized design (CRD), and Snedecor and Cochran's (1994) [14] analysis of variance was used to examine the data pertaining to various parameters that were gathered during the current study

Results and Discussions

Nutrient composition of *Azolla* leaf powder

The data revealed that *Azolla* leaf powder (ALP) contained 89.73, 23.49, 3.7, 14.7, 33.84, 24.26 percent dry matter, crude protein, ether extract, crude fibre, nitrogen free extract and total ash, respectively which is close agreement with the findings Lakshmi *et al.* (2019) [16]. Paudel *et al*. (2015) [21] also reported similar values for dry matter, crude protein and ether extract as 89.73, 23.49 and 3.7, respectively. The CP content of *Azolla* leaf powder in the present study was estimated as 23.49 per cent which is consistent with the results Sharma *et al*. (2020) [33] who reported CP content of 23-25%. However, the present result was contradicted with Parthasarathy *et al.* (2002) [20] and Shamna *et al.* (2013) [32] who reported 25 to 28% CP, which were higher than the current finding. The value of EE (3.7%) of DAM obtained in the present study is in harmony with the values reported by previous workers Sharma *et al*. 2020 [33]. Whereas, lower EE values ranging from 1.60 to 2.93% were reported in previous studies (Parthasarathy *et al*., 2002 and Shamna *et al*., 2013) [20, 32]. The CF content of 14.3% is in agreement with the findings of Parthasarathy *et al.* (2002) [20] reported that NFE content of the fern varies between 38.85 to 44.06%, however, in our study NFE value of 33.84% was recorded. The total ash content of 18.1% was recorded which corroborates with the findings. However, Shukla *et al*. (2018) [34] reported values of dry matter, crude protein, crude fibre, ether extract and total ash to be 98.8, 25.64, 17.29, 3.15 and 21.67 percent, respectively. The wide variations were existed in the chemical composition of dried *Azolla* meal (DAM), which might be due to differences in the *Azolla* strains, methods of cultivation and harvesting, methods of collection and processing, environmental conditions such as temperature, humidity, wind velocity and light intensity as well as nutrient profiling of soil, water and cow dung slurry which consequently affect *Azolla* plants' growth morphology and nutrient composition. Furthermore, contamination with dirt, debris, fallen tree leaves and other epiphytic algae could also be important factors which affect the nutrient composition of dried *Azolla* meal (Sharma *et al*., 2020) [33].

Nutrient utilization

The information on nutrient usage is shown in Table 1. The dry matter intake (DMI) in Kadaknath chicken was 68.94±1.75, 65.60±1.14, 76.57±1.17, 66.23±1.31 and 72.05±1.17 g in T_1, T_2, T_3, T_4 and T_5, respectively. The dry matter intake was significantly ($p<0.05$) highest in T_3 as compared to rest of the treatment groups. Significantly lowest DMI was found in T_2 and T_4 as compared to rest of treatment groups except T_1. The difference between T_1, T_5; T_1, T_2, T_4 was found non-significant. The digestible DMI was 45.58±1.68, 43.08±0.97, 55.89±1.13, 44.63±0.79 and 49.39±0.43 g in T_1, T_2, T_3, T_4 and T_5, respectively. The digestible DMI was significantly highest in T_3 and lowest digestible DMI was observed in T_1, T_2 and T_4. The difference between T_1, T_2 and T_4 was found non-significant. The crude protein intake (CPI) was 11.35±0.28, 10.99±0.19, 12.41±0.19, 10.67±0.21 and 11.62±0.19 g in T_1, T_2, T_3, T_4 and T_5, respectively. The CPI was significantly highest in T_3 as compared to rest of the treatment groups. Significantly lowest crude protein intake was found in T_2 as compared to rest of the treatment groups except T_1. The difference between T_1, T_5 as well as between T_1, T_2, T_4 was found to be non-significant. The digestible CPI was 7.71±0.28, 7.47±0.16, 8.98±0.18, 7.67±0.28 and 8.08±0.11 g in T_1, T_2, T_3, T_4 and T_5, respectively. The digestible CPI was significantly highest in T_3 as compared to rest of the treatment groups. The difference between T_1, T_2, T_4 and T_5 was found non-significant. The crude fibre intake (CFI) was 4.68±0.11, 4.45±0.07, 5.20±0.08, 4.50±0.08 and 4.89±0.08 g in T_1, T_2, T_3, T_4 and T_5, respectively. The CF intake was significantly highest in T_3 as compared to rest of the treatment groups and lowest crude fibre intake was found in T_2 as compared to rest of the treatment groups except T_4. The difference between T_1, T_4 and T_5 as well as between T_2 and T_4 was found non-significant. The digestible CF intake was 3.21±0.09, 3.05±0.04, 3.62±0.03, 3.10±0.05 and 3.35±0.07 g in T_1, T_2, T_3, T_4 and T_5, respectively. The digestible CF intake was significantly highest in T_3 and lowest value was found in T_2 and T_4 as compared to rest of the treatment groups expect control group. The difference between T_1 and T_5 as well as between T_1, T_2

and T_4 was found to be non-significant. The ether extract intake was 3.01±0.07, 2.95±0.05, 3.41±0.05, 2.90±0.05 and 3.16±0.05 g in T_1, T_2, T_3, T_4 and T_5, respectively. The ether extract intake was significantly highest in T_3 whereas lowest ether extract intake was found in T_4 as compared to rest of the treatment groups expect T_2. The difference between T_1, T_2 and T_5 as well as between T_2 and T_4 was found non-significant. The digestible ether extract intake was 2.17±0.04, 2.14±0.03, 2.52±0.05, 2.17±0.05 and 2.38±0.05 g in T_1, T_2, T_3, T_4 and T_5, respectively. The digestible ether extract intake was significantly highest in T_3 followed by T_5 and lowest digestible ether extract intake was found in T_1, T_2 and T_4. The difference between T_1, T_2 and T_4 was found non-significant. The NFE intake was 46.50±1.18, 44.24±0.77, 51.65±0.79, 44.67±0.88 and 48.60±0.79 g in T_1, T_2, T_3, T_4 and T_5, respectively. The NFE intake was significantly highest in T_3 followed by T_5 and lowest NFE intake was found in T_1, T_2 and T_4. The difference between T_1, T_2 and T_4 was found non-significant. The digestible NFE intake was 30.47±0.95, 29.22±0.44, 37.09±0.86, 29.03±0.72 and 31.57±0.70 g in T_1, T_2, T_3, T_4 and T_5, respectively. The digestible NFE was significantly highest in T_3 and significantly lowest digestible NFE intake was found in T_2 and T_4 as compared to rest of the treatment groups except T_1. The difference between T_1, T_5 as well as between T_1, T_2 and T_4 was found non-significant.

Table 1: Effect of feeding *Azolla* leaf powder on nutrient intake (g/birds/day) and utilization in Kadaknath chicken

Parameters/Treatments	T_1	T_2	T_3	T_4	T_5	SEm±	CD at 5%
Dry matter intake (g/bird/day)	68.94^{bc}±1.75	65.60^{c}±1.14	76.57^{a}±1.17	66.23^{c}±1.31	72.05^{b}±1.17	1.03	3.25
Digestible DMI (g/bird/day)	45.58^{c}±1.68	43.08^{c}±0.97	55.89^{a}±1.13	44.63^{c}±0.79	49.39^{b}±0.43	0.93	2.93
Crude protein intake (g/bird/day)	11.35^{bc}±0.28	10.99^{c}±0.19	12.41^{a}±0.19	10.67^{c}±0.21	11.62^{b}±0.19	0.16	0.53
Digestible Crude protein intake (g/bird/day)	7.71^{b}±0.28	7.47^{b}±0.16	8.98^{a}±0.18	7.67^{b}±0.28	8.08^{b}±0.11	0.17	0.54
Crude fibre intake (g/bird/day)	4.68^{b}±0.11	4.45^{c}±0.07	5.20^{a}±0.08	4.50^{bc}±0.08	4.89^{b}±0.08	0.07	0.22
Digestible Crude fibre intake (g/bird/day)	3.21^{bc}±0.09	3.05^{c}±0.04	3.62^{a}±0.03	3.10^{c}±0.05	3.35^{b}±0.07	0.05	0.18
Ether extract intake (g/bird/day)	3.01^{b}±0.07	2.95^{bc}±0.05	3.41^{a}±0.05	2.90^{c}±0.05	3.16^{b}±0.05	0.04	0.14
Digestible ether extract intake (g/bird/day)	2.17^{c}±0.04	2.14^{c}±0.03	2.52^{a}±0.05	2.17^{c}±0.05	2.38^{b}±0.05	0.04	0.13
Nitrogen free extract intake (g/bird/day)	46.50^{c}±1.18	44.24^{c}±0.77	51.65^{a}±0.79	44.67^{c}±0.88	48.60^{b}±0.79	0.6	2.20
Digestible NFE Intake (g/bird/day)	30.47^{bc}±0.95	29.22^{c}±0.44	37.09^{a}±0.86	29.03^{c}±0.72	31.57^{b}±0.70	0.60	1.92

Means with the same superscript in a particular row do not differ significantly (p<0.05) from each other.

The data pertaining to digestibility coefficients and nutrient balances in Kadaknath chicken in different treatments are presented in Table 2. The digestibility coefficient of dry matter was 66.08±1.15, 65.70±1.67, 72.98±0.57, 67.43±1.53 and 68.56±0.71 per cent in T_1, T_2, T_3, T_4 and T_5, respectively. The digestibility coefficient of DM was significantly highest in T_3 as compared to rest of the treatment groups. The difference between T_1, T_2, T_4 and T_5 was found non-significant. The digestibility coefficient of crude protein ranged between 67.91±1.20 to 72.32±0.57 per cent among different treatment groups. The difference in digestibility coefficient of CP in different treatments were small and found statistically non-significant. The digestibility coefficient of crude fibre ranged between 68.45±0.58 to 69.65±0.66 per cent among different treatment groups. The difference in digestibility coefficient of CF in different treatments were small and statistically non-significant. The digestibility coefficient of ether extract was 72.34±0.58, 72.70±0.46, 73.91±0.63, 74.88±0.60 and 75.53±0.57 per cent in T_1, T_2, T_3, T_4 and T_5, respectively. The digestibility coefficient of ether was significantly highest in T_4 and T_5 as compared to rest of the treatment groups except T_3 and lowest in T_1 and T_2 as compared to rest of the treatment groups except T_3. The difference between T_3, T_4 and T_5 as well as between T_1, T_2 and T_3 was found non-significant. The digestibility coefficient of NFE was 65.51±0.63, 66.05±0.52, 71.79±0.58, 64.98±0.58 and 64.95±0.59 per cent in T_1, T_2, T_3, T_4 and T_5, respectively. The digestibility coefficient of NFE was significantly highest in T_3 as compared to rest of the treatment groups. The difference between T_1, T_2, T_4 and T_5 was found non-significant. The nitrogen intake was 1.81±0.04, 1.75±0.03, 1.98±0.03, 1.70±0.03 and 1.86±0.03 g in T_1, T_2, T_3, T_4 and T_5, respectively. The nitrogen intake was significantly highest in T_3. Nitrogen intake was found significantly lowest in T_4 as compared to rest of the treatment groups except T_2. The difference between T_1, T_5; T_1, T_2 and T_2, T_4 was found non-significant. All the birds in different treatments groups were in positive nitrogen balance. The nitrogen balance (g/bird/day) was 1.23±0.04, 1.19±0.02, 1.43±0.03, 1.22±0.03 and 1.29±0.02 in T_1, T_2, T_3, T_4 and T_5, respectively. The nitrogen balance was significantly highest in T_3 as compared to rest of the treatment groups. Significantly lowest nitrogen balance was found in T_2 as compared to rest of the treatment groups except T_1 and T_4. The difference between T_1, T_4 and T_5 as well as between T_1, T_2 and T_4 was found statistically non-significant.

The results of present investigation revealed that DM, CP, CF, EE, NFE intake and digestibility coefficient of DM and NFE was significantly highest in group of birds fed diets containing 5% ALP as compared to other treatment groups. Non-significant effect was found in digestibility coefficient of CP, CF and EE among different treatment groups. Nitrogen Intake (g/bird/day) was highest in T_3 group (5% *Azolla*).

Table 2: Effect of feeding *Azolla* leaf powder on digestibility coefficient and Nutrient balance in Kadaknath chicken

Parameters/Treatments	T_1	T_2	T_3	T_4	T_5	SEm±	CD at 5%
Dry matter digestibility coefficient (%)	66.08^{b}±1.15	65.70^{b}±1.67	72.98^{a}±0.57	67.43^{b}±1.53	68.56^{b}±0.71	1.19	3.76
CP digestibility coefficient (%)	67.91±1.20	68.01±1.73	72.32±0.57	71.86±1.74	69.52±0.57	1.18	NS
CF digestibility coefficient (%)	68.49±0.58	68.45±0.58	69.65±0.66	69.01±0.58	68.55±0.57	0.48	NS
EE digestibility coefficient (%)	72.34^{b}±0.58	72.70^{b}±0.46	73.91^{ab}±0.63	74.88^{a}±0.60	75.53^{a}±0.57	0.56	1.78
NFE digestibility coefficient (%)	65.51^{b}±0.63	66.05^{b}±0.52	71.79^{a}±0.58	64.98^{b}±0.58	64.95^{b}±0.59	0.53	1.66
Nitrogen intake (g/bird/day)	1.81^{bc}±0.04	1.75^{cd}±0.03	1.98^{a}±0.03	1.70^{d}±0.03	1.86^{b}±0.03	0.03	0.08
Nitrogen balance (g/bird/day)	1.23^{bc}±0.04	1.19^{c}±0.02	1.43^{a}±0.03	1.22^{bc}±0.03	1.29^{b}±0.02	0.03	0.09

Means with the same superscript in a particular row do not differ significantly (p<0.05) from each other.

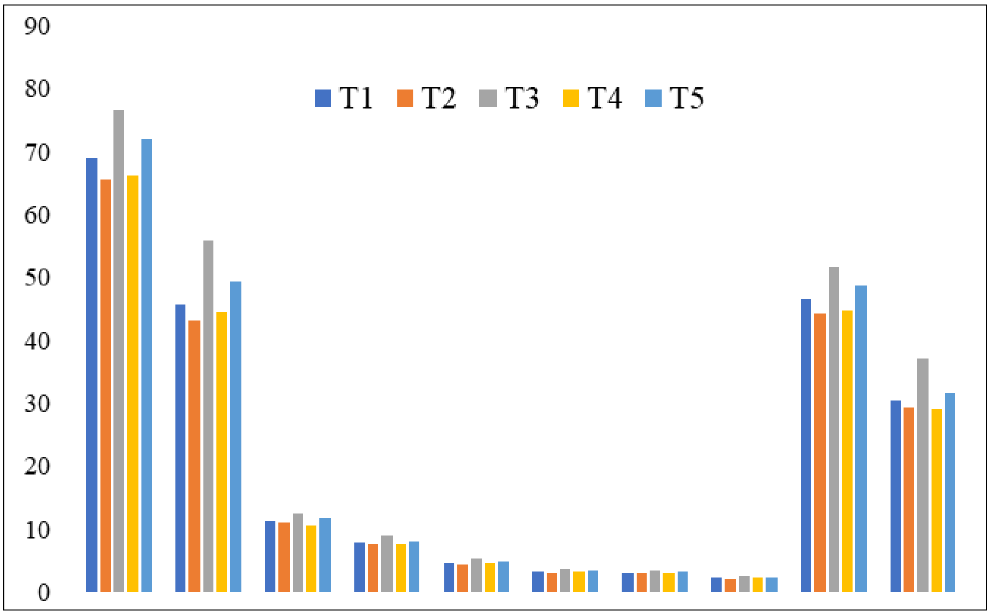

Fig 1: The difference between T_1, T_4 and T_5 as well as between T_1, T_2 and T_4 was found statistically non-significant

Nitrogen balance (g/bird/day) was positive in all birds in different groups. However, maximum Nitrogen balance was found in T_3 group (5% *Azolla*).The results of present study are in agreement with the findings of Mishra *et al.* (2016) [17] who reported that the metabolizable of crude protein was significantly higher in all treatment groups fed (5, 7.5 and 10% *Azolla* meal) versus the control group. The ability to break down dry materials was similar across all groups. In comparison to control groups, increased body weight growth and superior FCR may be associated with high protein retention in *Azolla*-fed groups. Similarly, Samad *et al*. (2020) [31] reported that feeding 10 and 15% of *Azolla* spp. led to significant improvement in nutrient digestibility. On other hand, Rathod *et al*. (2013) [26] found that adding *Azolla* at 5% level had no statistically significant impact on dry matter digestibility, however adding *Azolla* at 7.5 and 10% level caused a drop in dry matter digestibility. The non-significant differences in nitrogen balance and dry matter digestibility between groups were similarly documented by Rana *et al.* (2017) [25]. Kumar *et al.* (2018a) [14] reported that *Azolla* supplementation had no effect on DM digestibility, N intake and N retention. However, they found that all broilers in various treatment groups were found to have positive nitrogen balance which is agreement with the present study.

Conclusion
From the results, it may be concluded that 5% inclusion level of *Azolla* leaf powder in the diets resulted in improved nutrient utilization of Kadaknath chicken.

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