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# The Nutritional Value of *Azolla caroliniana* Wild as Animal Feed

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## Abstract

This research was conducted to investigate the nutritional value of *Azolla caroliniana* wild and its potential use as an alternative feed for animals. For this purpose, *Azolla caroliniana* was cultivated, harvested, and dried. The dried *Azolla* sample underwent proximate analysis. The dry matter content of *Azolla caroliniana* was 4.8%. The analysis of dry matter revealed 86.93% organic matter. Among them, 24.8% crude protein, 4.3% ether extract, 14.5% crude fiber and 41.32% nitrogen-free extract. According to the mineral composition of *Azolla*, it was found that there were 3.89% potassium, 1.89% calcium, 0.58% sulfur, 0.42% phosphorus, and 0.1456% iron, among other minerals. Chemical analyses showed that *Azolla caroliniana* is a plant rich in crude protein, microminerals, and vitamins. Thus, *Azolla caroliniana* can be regarded as a viable non-traditional feed for livestock.

## Keywords

*Azolla caroliniana*, Biochemical Composition, Microelements, Nutritional Value, Supplementary Feed for Animals, Protein Source

## 1. Introduction

Achieving high productivity and optimizing the physiological status of livestock are directly dependent on the development of a balanced and nutritionally adequate feeding system. In modern animal husbandry, the diversification of feed resources and the effective utilization of alternative nutritional sources remain critical scientific and practical challenges [1]. Traditional feedstuffs, including cereal-based feeds, hay, and protein-rich legumes, are often characterized by deficiencies in essential nutrients. In particular, conventional feeds may fail to provide adequate levels of digestible protein, essential amino acids (e.g., lysine and methionine), and bioavailable microelements (e.g., iron, zinc, and selenium), which are

crucial for livestock growth, immune function, and overall productivity [2] [3].

A deficiency of essential amino acids, particularly lysine and methionine, can disrupt nitrogen balance, impair protein synthesis, and consequently reduce growth rates and overall physiological performance in livestock [4] [5]. Similarly, inadequate levels of key microelements such as iron, zinc, and selenium can weaken the immune system, lower resistance to infections, and contribute to metabolic dysfunctions [6]. Nutritional imbalances in feed may also negatively impact reproductive performance, leading to decreased fertility rates and suboptimal offspring quality [7]. To address these challenges, the incorporation of high-protein plant-based feed additives into animal diets has garnered significant scientific interest. Among the most promising alternative feed sources is *Azolla caroliniana*, a rapidly growing aquatic fern with a high protein content (25% - 30%), a rich profile of essential amino acids, B vitamins, and a variety of microelements [8] [9]. The considerable concentrations of lysine and methionine in *Azolla caroliniana* enhance its potential as an effective protein supplement [10]. Furthermore, its symbiotic association with nitrogen-fixing cyanobacteria contributes to natural nitrogen enrichment, improving the overall nutritional value of feed formulations [3].

Despite its promising nutritional composition, the utilization of *Azolla caroliniana* as a livestock feed remains a subject of scientific debate. One of the primary concerns is the presence of antinutritional factors, such as tannins, phytates, and cyanogenic compounds, which may negatively affect digestion and nutrient bioavailability [11] [12]. Additionally, the biochemical composition of *Azolla caroliniana* is highly dependent on its growing conditions, water quality, and mineral availability, leading to variability in its nutritional profile. This necessitates careful consideration when incorporating it into animal feed formulations to ensure consistency and optimal dietary balance [13]. Given these factors, a comprehensive assessment of *Azolla caroliniana*'s role in mitigating nutritional deficiencies in conventional feeds and its overall applicability in livestock nutrition is essential. This study investigates the biochemical composition of *Azolla caroliniana* and explores its potential as a cost-effective livestock feed. We hypothesize that *Azolla caroliniana* contains sufficient crude protein and essential microelements to serve as a cost-effective and nutritionally viable alternative to traditional feed sources.

## 2. Methods

This research was conducted at the Vivarium Laboratory of the Institute of Biochemistry of Samarkand State University, the Department of Organic Chemistry, and the Research Institute of Plant Product Processing. The research aimed to investigate the effects of nutrient composition on the biomass formation dynamics of *Azolla caroliniana* and to evaluate its biochemical and microelement composition.

For *Azolla caroliniana* cultivation, experiments were carried out in outdoor containers with a water depth of 15 - 20 cm and a surface area of 1 $m^2$. The nutrient

medium contained decomposed cattle manure (5 g/L) and potassium nitrate ($KNO_3$) (2 g/L) dissolved in water. Each experimental unit was inoculated with 300 g of fresh *Azolla caroliniana* biomass. Environmental conditions were maintained at optimal levels, with temperatures ranging from 15˚C - 28˚C, a pH of 6.5 - 7.5, and a light intensity of 400 - 450 W/m$^2$. To ensure standardization and minimize external contamination, *Azolla caroliniana* was also cultivated in a controlled hydroponic system under identical conditions. After a 7-day growth cycle, *Azolla* caroliniana was harvested under controlled conditions. To prevent contamination, all samples were washed with deionized water and dried at 80˚C for 12 hours to achieve constant weight. Three independent batches (n = 5) were analyzed to minimize variability. Elemental composition was determined using atomic absorption spectrophotometry (AAS) based on the method [14] ensuring high precision and reproducibility. To validate the reliability of the results, each measurement was conducted in triplicate. The dried material was finely ground using a high-speed laboratory grinder and stored in hermetically sealed conditions for subsequent biochemical and elemental analyses. For trace element analysis, five independent batches of *Azolla caroliniana* (n = 5) were collected, each consisting of 200 g of dried biomass. The samples underwent acid digestion using a mixture of concentrated nitric acid ($HNO_3$) and hydrogen peroxide ($H_2O_2$) in a closed digestion system. The digested solutions were filtered and diluted with deionized water before analysis. Elemental concentrations of iron (Fe), calcium (Ca), potassium (K), and zinc (Zn) were measured using atomic absorption spectrophotometry (AAS) [14]. Measurements were conducted at wavelengths of 248.3 nm for Fe, 422.7 nm for Ca, 766.5 nm for K, and 213.9 nm for Zn. Internal standards were used for calibration, and concentrations were expressed in parts per million (ppm) on a dry mass basis.

The biochemical composition of *Azolla caroliniana* was analyzed following standardized laboratory protocols. The dry matter content was determined by drying the samples in an oven at 105˚C for 24 hours until a stable weight was achieved. Crude protein concentration was measured using the Dumas combustion method, an accurate and environmentally friendly alternative to the Kjeldahl method [15]. Lignin content was assessed using the Van Soest detergent fiber method, enabling differentiation between structural carbohydrates. Carbohydrate content was estimated by subtracting the sum of protein, lipid, ash, and fiber fractions from the total dry matter. The total energy value of the biomass was determined via bomb calorimetry, with results expressed in kilocalories per gram (kcal/g) (**Figure 1**).

A microscopic examination of *Azolla* caroliniana's tissue structure was performed to assess its cellular organization. Samples were fixed in FAA solution (formalin-acetic acid-alcohol), dehydrated through a graded ethanol series, and embedded in paraffin. Thin sections (5 - 7 μm) were prepared using a rotary microtome and stained with safranin-fast green for histological observation under a light microscope. This analysis provided insights into the anatomical adaptations

of *Azolla* caroliniana for nutrient absorption and metabolic activity. All experimental data were statistically analyzed using SPSS. The Shapiro-Wilk test was applied to assess data normality. A Student's t-test was used to compare mean values between the experimental and control groups. Results were expressed as mean ± standard deviation (SD), with statistical significance set at $p < 0.05$. Data visualization was performed using bar charts and scatter plots generated in OriginPro 2022 software to facilitate result interpretation and comparative analysis. This methodological framework ensures the reproducibility of findings and provides a comprehensive assessment of *Azolla caroliniana* as a sustainable, high-protein feed supplement.

(a) (b)

**Figure 1.** 7-day biomass of *Azolla caroliniana.*

## 3. Results

The biochemical analysis of *Azolla* caroliniana showed a dry matter content of 4.8%, with organic matter constituting 86.93%. The crude protein content was 24.8%, while ether extract was 4.3%, crude fiber was 14.5%, and nitrogen-free extract (NFE) made up 41.32% of the total composition (**Figure 2**).

These results align with findings from [8] [16], which reported crude protein content in *Azolla* pinnata ranging between 25% - 30%, suggesting that *Azolla* caroliniana has a comparable nutritional profile. However, the NFE content in our study (41.32%) was slightly lower than the values reported by [17], (47% - 47.4%), which may be attributed to differences in cultivation conditions and nutrient availability. The graphical representation (**Figure 2**) highlights that crude protein and nitrogen-free extract constitute the major components, underscoring *Azolla*'s potential as a high-protein feed ingredient. Statistical analysis (Student's t-test) confirmed significant differences in crude protein levels compared to traditional feed ingredients ($p < 0.05$).

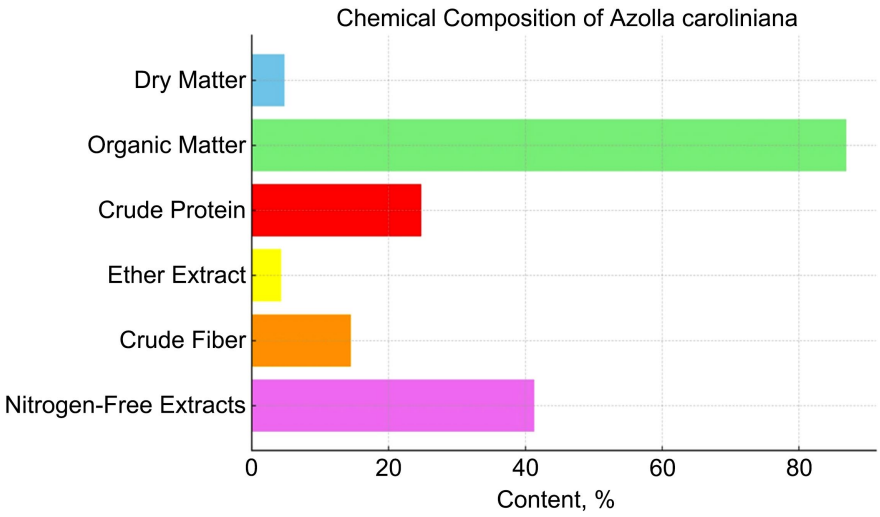


**Figure 2.** Chemical composition of *Azolla caroliniana.*

The microelement composition of *Azolla caroliniana* not only highlights its high nutritional value but also underscores its physiological adaptability and ecological resilience. As an aquatic plant, *Azolla caroliniana* efficiently absorbs and accumulates essential bioelements from its aquatic environment, contributing to its role as a sustainable and nutrient-rich feed source. The presence of vital microelements such as Fe, Zn, Mn, and Cu further highlights its potential as a functional feed additive, supporting metabolic processes and overall animal health. These characteristics make *Azolla caroliniana* a valuable biological resource in both agricultural and ecological applications (**Figure 3**).

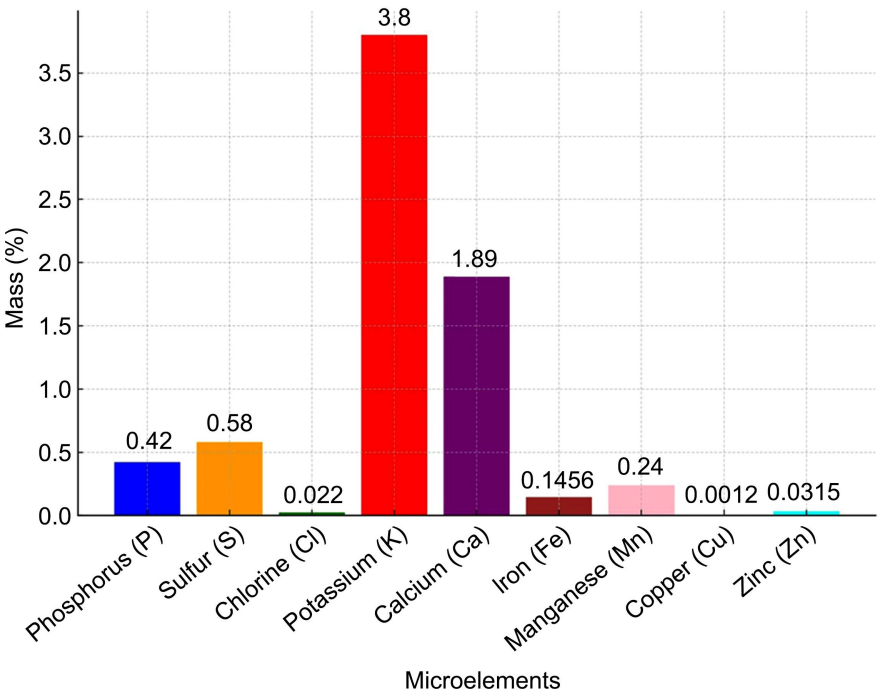


**Figure 3.** Microelement composition of *Azolla caroliniana* (%).

Figure 3 shows the microelement composition of *Azolla*, according to which the highest concentrations correspond to potassium (K)—3.8%, calcium (Ca)—1.89% and sulfur (S)—0.58%, which play an important role in ensuring cell ion exchange, osmotic balance and metabolic stability. Phosphorus (P)—0.42%, manganese (Mn)—0.24%, iron (Fe)—0.1456% are found in moderate amounts, which are involved in energy metabolism and the activation of fermentative processes. Zinc (Zn)—0.0315%, chlorine (Cl)—0.022% and copper (Cu)—0.012% are found in minimal concentrations.

The energy dispersive X-ray spectrum (EDX) presented in Figure 4 confirms the presence of major trace elements in *Azolla caroliniana*. The characteristic lines of the elements are reflected in the spectrum according to their X-ray intensity and energy levels. It was observed that the mineral composition of *Azolla* obtained in this study is almost similar to the values [17] [18].

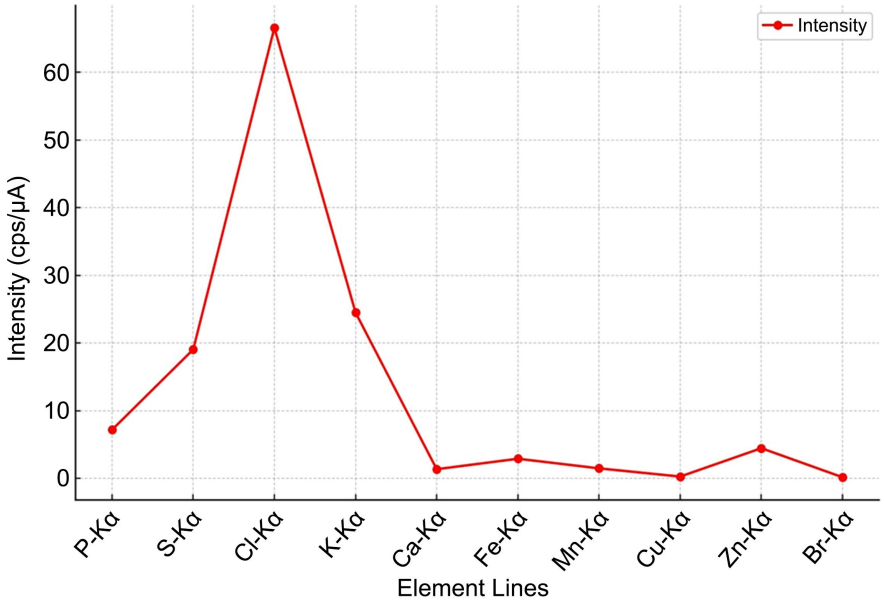


Figure 4. Elemental spectrum intensity.

The data were statistically analyzed using SPSS 27 software, and the Shapiro-Wilk normality test confirmed a normal distribution. Group comparisons were conducted using Student's t-test, with results expressed as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$. The statistically significant differences ($p < 0.05$) observed in *Azolla*'s biochemical and mineral composition suggest its substantial impact on livestock nutrition.

## 4. Discussion

The findings of this study indicate that *Azolla caroliniana* contains 24.8% crude protein, making it a valuable protein-rich feed component. Our findings are consistent with [8] [16], who reported crude protein levels of 25% - 30% in *Azolla pinnata*. However, variations in nitrogen-free extract (NFE) content may be at-

tributed to differences in water quality, pH levels, and nutrient availability [8]. The high potassium (3.89%) and calcium (1.89%) concentrations in our samples support the role of *Azolla caroliniana* in enhancing bone mineralization in livestock [13]. Nevertheless, the presence of antinutritional factors (tannins, phytates) should be carefully considered, as [2] reported potential digestibility issues in monogastric animals. In addition, the nitrogen-free extract (NFE) content (41.32%) was lower than the 47% - 47.4% reported by [17]. This discrepancy may be due to differences in fertilization methods, water quality, and environmental conditions, as observed in [19], as demonstrated that *Azolla*'s NFE content is directly influenced by nitrogen fertilization. Additionally, the calcium (1.89%) and phosphorus (0.42%) contents support its role in enhancing bone mineralization in livestock. These findings are consistent with those of [13], who reported similar calcium (1.85% - 1.92%) and phosphorus (0.4% - 0.5%) levels in *Azolla* species. Despite its nutritional advantages, the inclusion of *Azolla* in livestock feed remains a subject of scientific debate due to the presence of antinutritional factors. *Azolla* contains tannins, phytates, and other secondary metabolites, which may negatively affect digestibility [2]. A study by [6] demonstrated that ducks fed with *Azolla* exhibited improved weight gain and feed conversion efficiency. However, in [5] it was reported that monogastric animals such as pigs and poultry might experience lower digestibility rates due to *Azolla*'s fibrous nature. This suggests that *Azolla* may be more suitable for ruminants, which possess microbial populations capable of breaking down complex fibers and mitigating antinutritional effects. Another limitation is the low dry matter content (4.8%), which may restrict its use as a standalone feed. *Azolla* should be incorporated into balanced feed formulations rather than being used as a sole diet component [3]. The nutrient profile of *Azolla caroliniana* is significantly influenced by its growing environment. Mandal et al. reported that water quality and fertilization levels directly impact *Azolla*'s mineral composition [10]. According to [17], potassium content in *Azolla* varies with water pH and fertilization levels, with optimal values observed at pH 6.5 - 7.5. Our study maintained this pH range, aligning with previous findings. Moreover, Anitha et al. demonstrated that *Azolla*'s growth rate is temperature-dependent, with the 15˚C - 28˚C range being optimal for biomass production [1]. Our study adhered to these conditions, which likely contributed to the stability of its biochemical composition.

## 5. Conclusions

This research confirms that *Azolla caroliniana* is a nutritionally rich and sustainable feed supplement for livestock, containing 24.8% crude protein and essential microelements such as potassium (3.89%), calcium (1.89%), and phosphorus (0.42%). Its rapid growth and minimal cultivation requirements make it a cost-effective alternative to conventional feed sources. Further research is needed to assess its digestibility and long-term impact on animal health, particularly in monogastric species. *Azolla caroliniana* contains 24.8% crude protein, essential amino

acids, and key microelements such as potassium (3.89%), calcium (1.89%), sulfur (0.58%), phosphorus (0.42%), and iron (0.1456%). These components play a crucial role in supporting optimal metabolic functions, strengthening the immune system, and enhancing growth efficiency in animals.

Furthermore, the high digestibility and favorable fiber content of *Azolla caroliniana* make it an effective supplement in livestock diets, improving feed conversion efficiency and overall productivity. Previous studies have shown that incorporating *Azolla* into poultry and ruminant feed accelerates weight gain, boosts immunity, and increases milk yield. Its rapid growth rate, minimal cultivation requirements, and ability to actively assimilate essential nutrients from aquatic environments highlight its potential as a cost-effective and sustainable alternative to conventional feed sources.

In conclusion, *Azolla caroliniana* is a valuable feed source for promoting sustainable livestock farming, reducing costs and supporting eco-friendly agriculture. Integrating *Azolla* caroliniana into livestock nutrition programs can significantly improve feed efficiency, lower feed costs, and enhance overall animal health and productivity.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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