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Influence of *Azolla filiculoides* on Dairy Cow Milk Production and Improvements in Macro Nutrients and Milk Quality Indicators

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ABSTRACT

The dairy industry in India plays a pivotal role in rural development, employment, and nutritional security, making the quality of milk produced a key factor for both consumer health and economic viability. Enhancing milk quality, particularly in terms of fat, protein, and casein content, is essential for the production of high-value dairy products. Azolla filiculoides, a nutrient-rich and sustainable feed supplement, has shown potential to significantly improve these milk quality parameters. In a study involving 20 lactating HF crossbreed cows, Azolla supplementation led to notable increases in milk yield, with improvements ranging from 13.70% to 30.89%. Milk fat content ranged from 2.49% to 4.98%, protein content increased from 2.63%-3.24% to 3.16%-3.98%, and casein levels improved from 2.01%-2.3% to 2.66%-3.04%. Minimal changes were observed in lactose and galactose levels, indicating that while Azolla enhances fat and protein content, it does not significantly alter milk sugars. These results highlight A. filiculoidesas an effective, sustainable feed supplement that enhances milk quality, supporting the dairy industry's objectives of improving nutritional outcomes and economic sustainability.

Keywords: Azolla filiculoides, Nutritional quality of milk, Milk composition analysis, Dairy cattle nutrition, Sustainable dairy farming.

I. INTRODUCTION

The dairy industry in India is a cornerstone of the country's socio-economic status, playing a crucial role in rural development, employment generation, and nutritional security. As the world's largest milk producer, India has seen significant advancements in milk production, which have profoundly shaped the livelihoods of millions, particularly in rural areas. This growth, supported by government initiatives like "Operation flood" and cooperative movements, has transformed the dairy landscape, contributing significantly to the nation's GDP and accounting for over 4% of the economy (Pandey et al., 2024; Kolhal, 2022). The dairy sector is also vital for international trade, with about 70% of Indian exports linked to

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agricultural products, including dairy (Ramani et al., 2023; Kolhal, 2022).Cattle play a multifaceted role in this industry, being integral to both food production and agricultural activities. They support rural livelihoods by being used for plowing fields and transporting goods, making them essential for agricultural productivity (Mahajan and Singh, 2022). Moreover, cattle are central to dairy production, providing a stable income for millions of dairy farmers across India. However, this reliance on cattle brings challenges related to sustainable development, as the over-exploitation of natural resources, such as land and water, for cattle rearing poses significant environmental issues (Mahajan and Singh, 2022).

Milk, as a primary product of this industry, plays a crucial role in the daily lives of Indians. It serves as a major source of essential nutrients, particularly for a largely vegetarian population, and its per capita availability in India is significantly higher than the global average, ensuring nutritional security for many (Pandey et al., 2024; Ramani et al., 2023). Milk also holds cultural significance in India, being a key component of various culinary traditions and dietary practices, with products ranging from milk to cheese and yogurt contributing to a balanced diet (Brown et al., 2022; Balasubramanian, 2024).

Feed supplements play a vital role in enhancing cattle nutrition, improving feed efficiency, productivity, and overall health. These supplements, ranging from probiotics to specific nutrient additives, are crucial in boosting milk yield and enhancing the nutritional value of milk (Hassen et al., 2022). However, normal feed often leads to nutritional imbalances, inefficiencies, and environmental impacts. Issues like low protein utilization and poor conversion rates contribute to reduced productivity and increased environmental pollution from nitrogen waste, highlighting the need for alternative, sustainable feed supplements (Kovalenko et al., 2024; Zhao, 2018). Addressing these challenges is essential for the continued growth and sustainability of India's dairy industry.

A. filiculoides, a species within the *Azolla* genus, is particularly noted for its versatile applications, including phytoremediation, biofuel production, and livestock feed supplementation. This species is highly effective in accumulating and degrading contaminants such as arsenic, heavy metals, and polycyclic aromatic hydrocarbons (PAHs). Studies have demonstrated up to 100% removal efficiency for copper and over 90% biodegradation rates for organic pollutants like naphthalene (Biswas and Ganesan, 2024; Abdulwahab et al., 2022; Zazouli et al., 2023; Kösesakal and Seyhan, 2022). In biofuel production, *A. filiculoides* serves as a promising feedstock, capable of being converted into bioethanol through optimized processes that achieve high alcohol yields, and it is also a potential source for biodiesel, meeting international standards through optimized transesterification processes (Christy et al., 2024; Ahmad et al., 2023; Sundararaman, et al., 2024).Additionally, *A. filiculoides*is a cost-effective feed resource, rich in amino acids, minerals, and vitamins, enhancing livestock productivity, particularly in regions facing feed shortages. Beyond its use in feeding, it contributes to soil fertility improvement and can be used in composting and biogas production, further expanding its agricultural benefits (Alebachew et al., 2024). The nutritional composition of *Azolla* includes a high protein content ranging from 21% to 30% dry matter, making it a valuable feed supplement comparable to conventional protein sources like soybeans and maize (Kumar et al., 2020; Nasir et al., 2022; Alebachew et al., 2024). In addition to its protein, *Azolla* contains essential fatty acids and active compounds such as phenolics and tannins, which enhance its nutritional profile (Nasir et al., 2022). When used as a supplementary feed, *Azolla's* high protein and nutrient content can significantly improve growth rates and productivity in livestock, including cattle (Nasir et al., 2022; Alebachew et al., 2024).

Azolla can be easily cultivated in water bodies, particularly in tropical and subtropical regions, providing a sustainable and low-cost feed resource for

cattle (Kumar et al., 2020; Alebachew et al., 2024). It is commonly used as a supplementary feed alongside other feed resources to enhance feed intake, growth, and milk yield in dairy cattle (Alebachew et al., 2024).

The aim of this study was to assess the impact of supplementing the diet of dairy cows with *A. filiculoides*on various milk quality parameters, including milk yield, fat content, protein percentage, and other compositional elements such as lactose, casein, and galactose.. The need for this study arises from the growing demand for sustainable and cost-effective dietary supplements that can enhance milk production and improve its nutritional quality, while also ensuring the safety of dairy products. As *A. filiculoides* is known for its rapid growth, high nutritional content, and environmental benefits, this study aimed to explore its potential as a valuable addition to dairy cow diets, contributing to both the economic viability of dairy farming and the nutritional quality of milk.

II. MATERIALS AND METHODS

Site Selection for *A. filiculoides* Cultivation

The cultivation of *A. filiculoides*was strategically established within Mr. Raghavendra Bhat's coconut plantation farm located in Mittlakatte, Davangere district. This site was deliberately chosen due to its convenient proximity to essential amenities such as the residence, water source, and road access, which facilitated regular maintenance and monitoring of the cultivation pond. To create an optimal environment for *A. filiculoides*growth, careful preparation was undertaken. The pond floor was meticulously leveled, with all potential obstacles such as stones, roots, and sharp objects removed to ensure a smooth and unobstructed surface. Consistent water levels were maintained throughout the pond to create an ideal habitat for *A. filiculoides*, promoting uniform growth. Additionally, shading was provided to minimize water evaporation, helping to conserve water and sustain the necessary conditions for the fern's healthy development.

Pond Construction and *A. filiculoides*Cultivation

For the cultivation of *A. filiculoides*, three carefully constructed cement concrete tanks were utilized, each measuring 6 feet in length, 3 feet in width, and 2 feet in depth. These tanks provided a stable and spacious environment conducive to the growth of *A. filiculoides*. A uniform layer of finely sifted fertile soil, about 10-15 centimeters thick, was mixed with cow dung and 5-6 kilograms of vermicompost, creating a nutrient-rich base. This mixture was then moistened to form a slurry, which was evenly spread across the pond floor. The water level in the pond was maintained at three-fourths capacity, with regular monitoring to ensure consistency. Following pond preparation, a pure culture of *A. filiculoides*sourced from Krishi Vigyan Kendra, Davangere, Karnataka, was introduced into the pond. Water temperature was carefully regulated between 20-30°C, and pH levels of both the organic matter at the bottom and the surface water were routinely tested to maintain optimal conditions. To control sunlight exposure and prevent contamination, a green net was installed over the pond. Systematic maintenance, including pest and disease management, was conducted to ensure the health of the *A. filiculoides* culture. Every six months, the pond was emptied, and a fresh culture was introduced to sustain optimal growth conditions.

Harvesting of *A. filiculoides*

Once inoculated under favorable environmental conditions, *A. filiculoides* typically completes its growth cycle within two weeks. Harvesting is conducted using a plastic tray equipped with a mesh sieve, which allows for efficient water drainage. After harvesting, the *A. filiculoides* undergoes thorough washing to eliminate any cow dung odor and to separate smaller plantlets that may pass through the sieve. The cleaned *A. filiculoides* is then weighed, sun-dried for 2-3 days, and stored in airtight bags to preserve its quality until needed.

Impact of *A. filiculoides* Supplementation on Milk Yield and Nutrient Utilization in Lactating Dairy Cows

The study involved 20 lactating HF crossbreed cows in their second to fourth lactation, sourced from Rajappa Naik, a farmer in Jarikatte, Davangere district (C1-C10 control; A1 to A10 *A. filiculoides* Feeded cows). These cows were selected to assess the impact of *A. filiculoides* supplementation on milk production and nutrient efficiency. Each cow received a daily supplement of 1 kg of *A. filiculoides*, cultivated at Mr. Raghavendra Bhat's coconut plantation farm in Mittlakatte, Davangere district, along with 2 kg of standard cow feed. Prior to introducing the *A. filiculoides* supplementation, the cows were monitored as control subjects for two months to establish baseline data on key milk components, which are essential for evaluating cow health, milk production, and overall dairy quality. This control phase provided a foundation for comparing the effects of *A. filiculoides* supplementation on the cows' milk yield and nutrient efficiency.

The study will measure key milk components, including fat, solids-not-fat (SNF), protein, and total solids (TS), all of which are critical indicators of milk quality and cow health. Fat provides essential energy for maintaining milk production and body condition, while SNF, comprising proteins, lactose, and minerals, contributes to overall milk quality. Protein is crucial for growth and consistent milk production, and TS reflects the dry matter content, indicative of higher milk quality. Milk density, influenced by fat and SNF, will be assessed to gauge richness and quality. Additional components such as galactose, casein and urea will also be evaluated to ensure milk safety and quality. These analyses will be conducted at Shivamogga Milk Union Limited (SHIMUL) in Harihara, Davangere district. During the two-month *Azolla* supplementation period, cows will be monitored daily for milk yield, housed in well-maintained shelters, and milked twice daily at 5 a.m. and 5 p.m. to ensure accurate data collection.

III.RESULTS AND DISCUSSION

The various stages and components involved in the cultivation and supplementation of *A. filiculoides* in dairy cow feed trials is depicted in figure1 (A-G). The pond is prepared for *A. filiculoides* cultivation with a levelled soil base.This is followed by the application of a cow dung slurry, which provides essential nutrients for the growth of *A. filiculoides*. The robust proliferation of *A. filiculoides* is evident 15 days post-inoculation, demonstrating its rapid growth. The HF cow breeds selected for the study are also featured, highlighting their role in the feeding trials. The harvested *A. filiculoides* is shown, ready to be used as a supplementary feed, emphasizing its potential as a nutrient-rich addition to the cows' diet.

Milk Yield

The comparison of cow milk yield between regular feeding and *A. filiculoides* supplementation aligns well with existing literature, highlighting the potential of *Azolla* as a beneficial dietary supplement in dairy cattle diets. In this study, *A. filiculoides* supplementation led to a significant increase in milk yield, with individual cows showing improvements ranging from 13.70% to 30.89%. Notably, cows C7, A4, and A9 exhibited the highest increases, with milk yields rising by 30.64%, 30.89%, and 29.03%, respectively (Figure 2). These findings are consistent with previous studies, such as those by Bhatt et al. (2020) and Acharya et al. (2023), which reported increases in milk yield by 15-20% when *Azolla* was included in the diet.

Similarly, Gowda et al. (2015) documented a 4.8% increase in milk yield with *Azolla* supplementation, while Kerketta et al. (2020) and Kantwa et al. (2022) reported increases ranging from 12.5% to 26.04% in various field trials. This study's higher percentages, particularly those over 25%, reflect the effectiveness of *A. filiculoides* compared to earlier research, suggesting that its use may be even more beneficial in certain dairy management practices. The high nutritional value of *Azolla*, which improves feed

efficiency and overall animal health, is likely responsible for these improvements in milk production.

Analysis of Macronutrients

The analysis of macronutrients in this study highlights the impact of *A. filiculoides* supplementation on milk composition, particularly in terms of fat, protein, and casein content, while demonstrating minimal effects on lactose and galactose levels.

Fat Percentage

The results showed a significant increase in milk fat content across all cows following the introduction of *A. filiculoides*. Prior to supplementation, the fat percentage ranged from 2.49% to 4.02%, which increased to 3.81% to 4.98% after supplementation. Notable increases were observed in cows C7, C9, A3, A4, and A9, with cow C7's fat percentage rising from 2.49% to 4.94%, nearly doubling its initial value. These findings suggest that *A. filiculoides* significantly enhances milk fat content, improving its nutritional quality and aligning with consumer preferences. This aligns with the findings of Kantwa et al. (2022), who reported a 4.76% increase in milk fat with *Azolla* supplementation, and up to 9.24% with additional feed enhancements. The nutrient-rich profile of *Azolla*, containing essential amino acids and minerals like calcium and phosphorus, supports milk fat production (Gowda et al., 2015; El-Naggar et al., 2022). Additionally, *Azolla*'s rapid growth and cost-effectiveness make it an appealing alternative to conventional feeds (Kthis et al., 2020).

Protein Percentage

The study also demonstrated a substantial increase in milk protein content after *A. filiculoides* supplementation, with the percentage rising from 2.63%-3.24% before supplementation to 3.16%-3.98% afterward. Significant increases were noted in cows C3, A2, A3, A6, and A8. For instance, cow C3's protein percentage increased from 3.03% to 3.94%, highlighting the effectiveness of *A. filiculoides* in enhancing the protein content of milk. These results are consistent with previous studies, such as those by Gowda et al. (2015) and Kantwa et al. (2022), which emphasize *Azolla*'s role in enhancing milk protein synthesis due to its high protein, amino acid, and mineral content. This increase improves the nutritional value and texture of dairy products like cheese and yogurt (Yasothai, 2014; Haque et al., 2013).

Casein Percentage

Casein content, essential for cheese production and overall milk quality, also showed a consistent increase following supplementation. Before the dietary intervention, casein percentages ranged from 2.01% to 2.3%, increasing to 2.66% to 3.04% after *A. filiculoides* was introduced. Significant improvements were observed in cows C7, C8, C9, and A3, with cow C9's casein content rising from 2.13% to 3.04%. These findings are aligned with previous studies that highlight *Azolla*'s ability to enhance casein content, making milk more suitable for dairy processing (Gowda et al., 2015; Kthis et al., 2020). The increase in casein further underscores *A. filiculoides*' potential as an effective supplement to improve milk quality (Huppertz, 2022).

Lactose Percentage

Slight increases were observed in lactose content, with the percentage rising from 2.01%-3.43% before supplementation to 2.07%-3.54% afterward. However, these changes were minimal and did not indicate a significant shift in lactose production. For example, cows C4, C5, and C10 showed negligible increases in lactose percentage. This aligns with existing literature suggesting that *Azolla* supplementation primarily enhances fat and protein without significantly impacting lactose, which plays a crucial role in energy provision, calcium absorption, and gut health (Mudgal et al., 2022; Huppertz, 2022; Guerville and Ligneul, 2024).

Galactose Percentage

The study also assessed galactose levels and found slight decreases in galactose content after *A. filiculoides* supplementation. Galactose percentages ranged from 0.09%-0.58% before supplementation and decreased slightly to 0.04%-0.24% afterward.

Minor changes were noted, with cows C1, C3, and C10 showing slight reductions in galactose percentage. This suggests that *A. filiculoides* does not significantly affect galactose content, maintaining its role in energy provision and cellular functions, as noted in previous studies (Rawat et al., 2015; Gowda et al., 2015; Sasaoka et al., 2018; Szilagyi, 2019).

Thus, the introduction of *A. filiculoides* into the cows' diet led to significant improvements in fat, protein, and casein content, while its effects on lactose and galactose were minimal (Figure 2). These results highlight *A. filiculoides* as an effective supplement for enhancing the nutritional profile of dairy milk, particularly in terms of fat and protein content, which are crucial for both dairy production and consumer preferences.

Milk Quality indicator

The comparison of milk quality indicators after *A. filiculoides* supplementation aligns with existing literature, demonstrating both improvements and stability in key components of milk.

SNF %

The SNF content, which includes proteins, lactose, minerals, and vitamins, is a critical indicator of milk quality and shelf life. This study observed a slight increase in SNF percentage from 7.16%-8.37% to 7.36%-8.57% following *A. filiculoides* supplementation. This modest change is consistent with previous research, which shows minimal impact of Azolla on SNF content. For example, Gowda et al. (2015), Mudgal et al. (2022), and Tirfie (2023) reported similar findings, indicating that Azolla supplementation improves milk yield without significantly altering the essential nutritional components like SNF. Additionally, Dubljević et al. (2020) found that variations in pasture quality can affect SNF levels, but Azolla maintains nutritional stability, ensuring the milk's quality.

TS %

The TS% in milk, which comprises fat, protein, lactose, and minerals, is vital for the nutritional value and processing characteristics of dairy products. In this study, *A. filiculoides* supplementation led to a slight increase in TS% from 9.55%-11.55% to 9.85%-11.85%. These changes, though minor, are in line with previous studies. Gowda et al. (2015) and Mudgal et al. (2022) similarly observed minimal impact on TS%, while Vyawahare et al. (2011) and Azevedo et al. (2023) noted that Azolla primarily enhances yield rather than significantly changing milk composition. This stability in TS% is important for preserving the milk's viscosity, stability, and sensory properties, which are crucial for dairy product acceptance among consumers.

Milk Density

Milk density, influenced by fat, protein, and mineral content, was observed to increase from 1.00-1.03 g/L to 1.02-1.30 g/L in this study. Cows A4 and C6 exhibited notable increases in density, which aligns with the literature suggesting that *Azolla* enhances milk fat content, a major determinant of milk density. Studies by Kantwa et al. (2022), Gowda et al. (2015), and Anitha et al. (2016) also support this relationship between higher fat content and increased milk density. The improvement in milk density observed in this study reflects *A. filiculoides*' positive impact on overall milk quality and nutritional value.

Urea Concentration

The urea content in milk serves as an indicator of protein metabolism and nutritional balance in dairy cows. In this study, urea levels showed minimal changes, with values ranging from 0.17-0.26 mg/L before supplementation to 0.16-0.25 mg/L afterward. These minor variations suggest that *A. filiculoides* has a neutral effect on protein metabolism, maintaining the balance of dietary protein without significant alterations. This finding is consistent with previous research, including studies by Onaciu et al. (2019), Guliński (2024), and Chen et al. (2017), which report stable milk urea levels as a sign of efficient protein utilization in dairy cows.

A. filiculoides offers a sustainable and safe option for dairy supplementation, preserving the nutritional and safety standards of milk. This aligns with efforts to

provide safer alternatives to harmful adulterants in the dairy industry.

IV. CONCLUSION

The findings from this study support the efficacy of *A. filiculoides*as a sustainable and effective dietary supplement for dairy cows, significantly enhancing key milk quality parameters such as fat, protein, and casein content. The introduction of *A. filiculoides* led to notable improvements in milk yield, with increases ranging from 13.70% to 30.89%, and substantially enhanced the nutritional profile of the milk. Importantly, the supplementation had minimal impact on lactose, galactose, and other milk sugars, indicating that *A. filiculoides* primarily boosts fat and protein content without disrupting the balance of essential milk components. Furthermore, the stability observed in lactic and citric acid levels, as well as the minimal changes in contaminant levels such as ammonium sulfate and melamine, underscores the safety of *A. filiculoides* as a feed supplement. These findings align with existing literature and highlight the potential of *A. filiculoides* to improve both the nutritional quality and safety of milk, making it a viable alternative to conventional feed supplements. Overall, the study demonstrates that *A. filiculoides* supplementation can contribute to enhanced milk production and quality, supporting the economic sustainability of dairy farming while ensuring the production of safe, high-quality milk. Future research should explore the long-term impacts of *A. filiculoides* on dairy production and its potential application across different dairy farming systems.

REFERENCES

[1]. Abdulwahab, I. A., Yasin, S. R., Jasim, S. S., Abdullah, S. R. S., Almansoory, A. F., & Ismail, N. I. 2022. Removal of copper by A. filiculoides and Lemna minor: Phytoremediation potential, adsorption kinetics, and isotherms. Heliyon, 8: e11456.

[2]. Acharya, P., Singh, Y., Sharma, A., Pattnaik, N., & Prakash, A. 2023. Azolla: An alternative feed for sustainable livestock production. International Journal of Veterinary Sciences and Animal Husbandry, 8: 10-16.

[3]. Ahmad, M. I., Senusi, W., Binhweel, F., &AlSaadi, S. A. 2023. Optimization of base catalytic transesterification toward maximum biodiesel yield from A. filiculoides macroalgae feedstock. Industrial Crops and Product, 197: 116590.

[4]. Alebachew, S., Zerihun, T., Nigussie, B., & Amsalu, F. 2024. Azolla as a beneficial macrophyte for livestock feed: A review. Cogent Food & Agriculture. 10: 2367804.

[5]. Anitha, K. C., Rajeshwari, Y. B., Prasanna, S. B., & Shilpa, J. 2016. Nutritive evaluation of Azolla as livestock feed. Journal of Experimental Biology and Agricultural Sciences, 4: 670-674.

[6]. Azevedo, R. A., Lage, C. F. A., Silper, B. F., DinizNeto, H. C., Quigley, J. D., & Coelho, S. G. 2023. Invited review: Total solids concentration in milk or milk replacer for dairy calves. Journal of Dairy Science, 106: 7341-7351.

[7]. Balasubramanian, G. 2024. Growth and development of dairy industry in India. European Economic Letters, 14: 451–462.

[8]. Bhat, M. Y., Dar, T. A., & Singh, L. R. 2016. Casein proteins: Structural and functional aspects. InTech. https://doi.org/10.5772/64187.

[9]. Bhatt, N., Singh, N. P., Singh, A., Kandpal, D., Chaudhary, P., &Patoliya, P. 2020. Azolla – A potent unconventional feed and its effect on feeding various livestock species: A review. Journal of Entomology and Zoology Studies, 8: 1693-1698.

[10]. Biswas, S., Mandal, A., Chakraborty, M., & Biswas, K. 2021. Determination of fat, SNF and protein content in cow milk from the voltage output of 'MilkTester'. I2MTC 2021 - IEEE

International Instrumentation and Measurement Technology Conference Proceedings. pp. 1-5.

[11]. Celik, B. 2022. Effect of citric acid on biofilm formed by P. fluorescens strains isolated from raw milk samples offered for consumption. International Journal of Pathogen Research, 9: 47-55.

[12]. Chaudhary, R., Rai, S., Sailo, L., Farooq, U. B., Singh, A., Naha, B. C., & Kumar, A. 2017. Genetic and non-genetic factors influencing fatty acid composition of dairy milk: A review. Indian Journal of Animal Nutrition, 34: 1.

[13]. Chen, J., Huang, M., Cao, F., Pardha-Saradhi, P., & Zou, Y. 2017. Urea application promotes amino acid metabolism and membrane lipid peroxidation in Azolla. PLOS ONE, 12: e0185230.

[14]. Christy, E., Wickramasinghe, I., Kapilan, R., &Wijesekara, I. 2024. Enhancing bioethanol production from A. filiculoides through optimization of pretreatment and culture conditions with Saccharomyces cerevisiae. Journal of Agricultural Sciences, 19: 171-187.

[15]. Chu, C. Y., & Wang, C. C. 2013. Toxicity of melamine: The public health concern. Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis & Ecotoxicology Reviews, 31: 342–386.

[16]. Dubljević, R., Marković, B., Radonjić, D., Stešević, D., &Marković, M. 2020. Influence of changes in botanical diversity and quality of wet grasslands through phenological phases on cow milk fatty acid composition. Sustainability, 12: 6320.

[17]. El-Naggar, S., & El-Mesery, H. S. 2022. Azollapinnata as unconventional feeds for ruminant feeding. Bulletin of the National Research Centre, 46: 66.

[18]. Fedorova, A., Kozlova, O., Slavyanskiy, A., Velichkovich, N., & Le, V. M. 2020. Research and development of maltodextrin production technology for the dairy industry. Food Processing: Techniques and Technology, 50: 616-629. 10.21603/2074-9414-2020-4-616-629

[19]. Gao, X., & Oba, M. 2016. Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. Journal of Dairy Science, 99: 291-300.

[20]. Gowda, N. K. S., Manegar, A., Shiv, S., Verma, N. C., Valleesha, G., Maya, G., Pal, D. T., & Suresh, K. P. 2015. Azolla (Azollapinnata) as a green feed supplement for dairy cattle: An on-farm study. Animal Nutrition and Feed Technology, 15: 283.

[21]. Guerville, M., &Ligneul, A. 2024. "Lactose: A Sugar Unlike Any Other." Cahiers de Nutrition et de Diététique, 59: 102-112. 59: 102-112.

[22]. Guliński, P. 2024. The effect of different content of protein and urea in milk, as biomarkers of energy-protein balance of food rations, on the level of selected milk performance characteristics of Polish Holstein-Friesian cows. Acta ScientiarumPolonorum, 34: 5-23.

[23]. Hanuš, O., Vegricht, J., Frelich, J., Macek, A., Bjelka, M., Louda, F., &Janů, L. 2018. Analysis of raw cow milk quality according to free fatty acid contents in the Czech Republic. Czech Journal of Animal Science, 53: 17-30.

[24]. Hassen, A., Ahmed, R., Alam, M., Chavula, P., Shek, S., &Dawid, A. 2022. The effect of feed supplementation on cow milk productivity and quality: A brief study. International Journal of Agriculture and Veterinary Sciences, 10: 13-25.

[25]. Huppertz, T. 2022. Lactose in milk: Properties, nutritional characteristics, and role in dairy products. In: Huppertz, T., & Vasiljevic, T. (Eds.), Understanding and improving the functional and nutritional properties of milk. BurleighDodds Science Publishing, pp. 361-380.

[26]. Kantwa, S. C., Kumar, U., & Patel, S. J. 2022. Azolla as a green fodder feed supplement of

cross-breed cows in district-Sehore (M.P.). Bhartiya Krishi AnusandhanPatrika, 36: 280-284.

[27]. Kerketta, S., Sarangdevot, S. S., Naruka, P. S., Verma, S., Pachauri, C. P., Singh, A. K., Singh, J. P., &Bhadauria, S. S. 2020. Effect of Azolla as feed supplement on milk production of lactating buffaloes at Neemuch district Madhya Pradesh. Indian Journal of Dairy Science, 73: 4.

[28]. Kolhal, M. T. 2022. Indian dairy industry. International Journal of Health Sciences (IJHS), 6: 10880–10885.

[29]. Kondrotienė, K., Zavistanavičiutė, P., Aksomaitienė, J., Novoslavskij, A., &Malakauskas, M. 2023. Lactococcus lactis in dairy fermentation—Health-promoting and probiotic properties. Fermentation, 10: 16.

[30]. Kösesakal, T., &Seyhan, M. 2022. Naphthalene stress responses of the aquatic fern A. filiculoides Lam. and evaluation of phytoremediation potential. Polycyclic Aromatic Compounds, 43: 6819-6836.

[31]. Kour, M., Khan, N., Singh, R., Mahajan, V., Amrutkar, S. A., & Kumar, D. 2020. Effect of Azolla (Azollapinnata) supplementation on milk yield, composition, and economics in crossbred HF cows. International Journal of Current Microbiology and Applied Sciences, 9: 2661-2666.

[32]. Kovalenko, V., Kovalenko, N., Gamayunova, V., Butenko, A. O., Kabanets, V., Salatenko, I., Kandyba, N., &Vandyk, M. 2024. Ecological and technological evaluation of the nutrition of perennial legumes and their effectiveness for animals. Journal of Ecological Engineering, 25: 294-304.

[33]. Kumar, U., Reager, M. L., &Mitharwal, B. S. 2020. Cultivation of Azolla (Azollapinnata) and its use as cattle feed supplement. International Journal of Current Microbiology and Applied Sciences, 9: 1174-1178.

[34]. Li, M. M., Huang, X., Campos, L. M., Prestegaard-Wilson, J. M., Pilonero, T., Budde, A., & Hanigan, M. D. 2023. Effects of dietary starch and ruminally undegraded protein on glucogenic precursors in lactating dairy cows. Animal,17(5): 100893.

[35]. Mahajan, V., & Singh, J. 2022. Lives, livelihoods and environment: The challenge of sustainable development goals in India. In: Antoniades, A., Antonarakis, A. S., &Kempf, I. (Eds.), Financial crises, poverty and environmental sustainability: Challenges in the context of the SDGs and Covid-19 recovery. Springer, Cham, pp. 169-185.

[36]. Mansour, H. A., Almatarneh, A. A., Abu-Saleh, A. A., &Elayan, I. A. 2017. Mechanistic and spectral investigation on the deamination of ammeline and ammelide. Computational and Theoretical Chemistry, 1117: 92-99.

[37]. Mathur, G. N., Sharma, R., & Choudhary, P. 2013. Use of Azolla (Azollapinnata) as cattle feed supplement. Journal of Krishi Vigyan, 2: 73-75.

[38]. Miciński, J., Zwierzchowski, G., Kowalski, I. M., &Szarek, J. 2012. Health-promoting properties of selected milk components. Journal of Elementology, 18: 165-186.

[39]. Mudgal, V. D., Jaiswal, P., Abraham, G., Kaushik, G. K., & Dahiya, S. S. 2022. Aquatic fern Azolla affecting milk production in buffaloes. Buffalo Bulletin, 41: 579-589.

[40]. Nasir, N. A. N. M., Kamaruddin, S. B. A., Zakarya, I. A., & Islam, A. K. M. A. 2022. Sustainable alternative animal feeds: Recent advances and future perspective of using Azolla as animal feed in livestock, poultry, and fish nutrition. Sustainable Chemistry and Pharmacy, 25: 100581.

[41]. Nayana, M. S., Deepak, N., Nisha, M., Shravani, M. S., & Manjunath, H. R. 2023. A review on milk quality detection and analysis. International Journal of Advanced Research in

Science, Communication and Technology (IJARSCT), 3: https://doi.org/10.48175/IJARSCT-7838.

[42]. Onaciu, G., Jurco, E., Jurco, S. G., Maciuc, V., &Ognean, L. 2019. Influence of varying ranges of milk urea nitrogen on chemical, hygienic, and physical quality traits of cow milk. Romanian Biotechnological Letters, 24: 866-873.

[43]. Oshima, M., & Fuse, H. 1981. Citric acid concentration in subclinical mastitic milk. Journal of Dairy Research, 40: 32-36.

[44]. Pandey, S., Mishra, S., Jha, A. K., & Pandey, H. O. 2024. An overview of recent dairy schemes revolutionizing the socio-economic landscape of India. International Journal of Agriculture Extension and Social Development, 7: 229-235.

[45]. Quiroz, D. A. A. 2022. Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. Foods, 11: 2276.

[46]. Ramani, A., Deshmukh, R., Seth, R., Gandhi, K., & Sharma, R. 2023. Development of dairy industry: An identity of India's self-reliance - A review. Bhartiya Krishi AnusandhanPatrika, 38: 203-209.

[47]. Rao, D. 2016. Safety of milk processing and distribution chain in India. In: Prakash, V., Martín-Belloso, O., Keener, L., Astley, S., Braun, S., McMahon, H., &Lelieveld, H. (Eds.), Regulating safety of traditional and ethnic foods. Academic Press, pp. 169-185.

[48]. Ravelo, A., Vyas, D., Ferraretto, L. F., &Faciola, A. P. 2022. Effects of sucrose and lactose as partial replacements for corn in lactating dairy cow diets: A review. Translational Animal Science, 6: 1-8.

[49]. Rawat, N., Kumari, K., Singh, F. V., &Gilhare, V. R. 2015. Effect of Azolla-supplemented feeding on milk production of cattle and production performance of broilers. Applied Biological Research, 17: 214.

[50]. Salman, M., El-Sayed, S. A., Al-Amoudi, M. S., Alghamdi, L. S., &Bazaid, S. A. 2012. Identification and determination of melamine in milk by high-performance liquid chromatography-UV detector. Scholars Research Library, 4: 737-748.

[51]. Sasaoka, N., Imamura, H., &Kakizuka, A. 2018. A trace amount of galactose, a major component of milk sugar, allows maturation of glycoproteins during sugar starvation. iScience,10: 211-221.

[52]. Sundararaman, S., Karthikeyan, M., Aravind Kumar, J., et al. 2024. Facile synthesis of iron nanoparticles from Camellia sinensis leaves catalyzed for biodiesel synthesis from A. filiculoides. Scientific Reports, 14: 12818.

[53]. Szilagyi, A. 2019. Digestion, absorption, metabolism, and physiological effects of lactose. In: Paques, M., & Lindner, C. (Eds.), Lactose. Academic Press, pp. 49-111.

[54]. Tirfie, F. W. 2023. A review of genetic and non-genetic parameter estimates for milk composition of cattle. Animal and Veterinary Sciences, 11: 64-70.

[55]. Vyawahare, A. S., Nawale, P., Kumar, S., Papinwar, K., Dhinalkumar, H. P., & Rao, K. J. 2011. Psychorheological study on viscosity of milk. Journal of Dairying, Foods and Home Sciences, 30: 1.

[56]. Yusa, K., Ando, K., & Onodera, Y. 1969. Citric acid content of cow's milk. Nihon ChikusanGakkaiho, 40: 32-36.

[57]. Zazouli, M. A., Ala, A., Asghari, S., &Babanezhad, E. 2023. Evaluation of A. filiculoides potential in pyrene and phenanthrene accumulation and phytoremediation in contaminated waters. International Journal of Phytoremediation, 26: 608-617.

[58]. Zhao, G. 2018. Improving feed protein utilization rate in cattle through nutritional

approaches. Current Protein & Peptide Science,
20: 164-171.



Figure 1: A= The pond is prepared for cultivating *A. filiculoides*; B= A uniform distribution of cow dung slurry across the pond surface provides essential nutrients for the growth of *A. filiculoides*; C= Significant proliferation of *A. filiculoides* is observed 15 days post-inoculation; D: HF cow breeds selected for the study; E: *A. filiculoides* utilized as a supplementary feed for the cows; F: Standard cow feed is administered alongside *A. filiculoides* during the feeding trials.

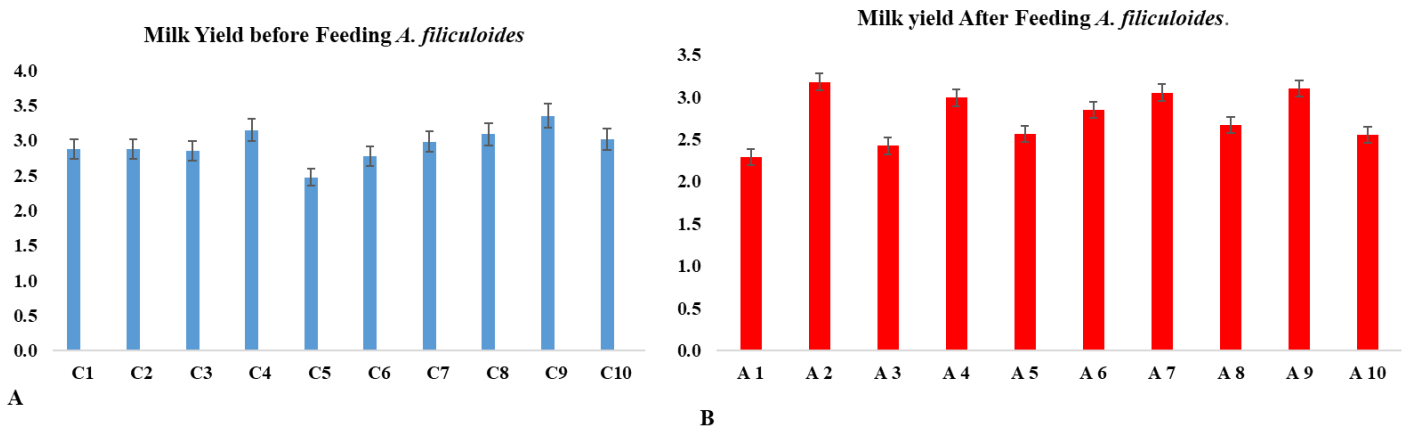


Figure 2: Effect of *A. filiculoides* supplementation on milk yield in 20 cows. A: Average milk yield (in liters) before feeding *A. filiculoides*, B: Average milk yield after feeding *A. filiculoides*. Each bar represents the mean value ± standard error of the mean (SEM), with data collected from three independent replicates per cow.

Table 1A: Milk Composition Parameters for Different Cows Before Intake of *A. filiculoides*

Milk Composition Parameters					
Milk Samples	Fat%	Protein%	Casein%	Lactose%	Galactose%
	Before Intake	Before Intake	Before Intake	Before intake	Before Intake
C1	3.4	2.66	2.13	2.27	0.12
C2	3.53	2.78	2.11	3.43	0.58
C3	4.02	3.03	2.01	3.06	0.22
C4	4.01	2.87	2.17	3.03	0.22
C5	3.08	3.23	2.19	2.24	0.23
C6	3.08	2.63	2.15	2.55	0.42
C7	2.49	2.92	2.18	2.01	0.09
C8	3.21	2.93	2.13	2.37	0.32
C9	3.77	2.69	2.16	2.02	0.21
C10	3.55	2.79	2.3	2.3	0.21

Table 1B: Milk Composition Parameters for Different Cows After Intake of *A. filiculoides*

Milk Composition Parameters					
Milk Samples	Fat%	Protein%	Casein%	Lactose%	Galactose%
	Before Intake	Before Intake	Before Intake	Before intake	Before Intake
A1	3.67	4.24	2.95	3.42	2.09
A2	3.71	4.55	3.19	3.92	2.05
A3	3.04	4.92	2.78	3.58	2.18
A4	3	4.89	2.96	3.93	2.16
A5	3.17	3.88	2.92	3.25	2.09
A6	3.8	4.03	2.94	3.43	2.05
A7	3.56	4.29	2.99	3.23	2.04
A8	3.08	3.86	3.24	3.91	2.07
A9	2.63	4.97	3.09	3.98	2.07
A10	3.78	4.39	3.01	3.98	2.16

Table 2A: Impact of *A. filiculoides* on various milk quality parameters in 10 cows before feeding.

Milk Samples	SNF%	TS%	Density g/L	Urea mg/L
	Before Intake	Before Intake	Before Intake	Before intake
C1	7.72	10.17	1.03	0.22
C2	7.52	9.73	1.03	0.22
C3	7.99	9.55	1.02	0.24
C4	8.19	10.41	1.02	0.21
C5	7.96	9.78	1.02	0.23
C6	7.16	10.5	1.02	0.21
C7	8	10.28	1.02	0.26
C8	8.23	10.59	1.03	0.26
C9	7.89	11.43	1.02	0.20
C10	7.97	11.55	1.02	0.18

Table 2B: Impact of *A. filiculoides* on various milk quality parameters in 10 cows After feeding.

Milk Samples	SNF%	TS%	Density g/L	Urea mg/L
	After Intake	After Intake	After Intake	After Intake
A1	8.27	10.57	1.23	0.22
A2	8.31	10.45	1.11	0.20
A3	7.51	10.53	1.10	0.17
A4	8.15	11.66	1.30	0.21
A5	7.85	11.65	1.20	0.22
A6	7.87	10.3	1.10	0.20
A7	8.08	10.48	1.20	0.18
A8	8.57	11.23	1.10	0.23
A9	8.22	10.75	1.10	0.16
A10	8.35	10.96	1.20	0.17