

Potentiality of *Eichhornia crassipes* as partial bovine feed: A step towards managing its invasiveness

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Abstract. Guchhait S, Paria DS, Shrestha R, Pradhan P, Roy A. 2025. Potentiality of *Eichhornia crassipes* as partial bovine feed: A step towards managing its invasiveness. *Asian J Agric* 9: 60-67. Menace of invasive species is rampant in tropical and subtropical areas and is a threat to the integrity of regional aquatic ecosystems. Bio-utilization has been suggested as one of the means of controlling the populations of invasive species. However, invasive plants such as Water Hyacinth (WH) (*Eichhornia crassipes*) are also known to accumulate heavy metals. From the literature survey, the young leaves of WH collected from freshwater ponds were found to have the lowest amounts of heavy metals in comparison to the roots as well as other fodder plants for bovines. This study aims to determine the effects of substituting common traditional bovine feed used in Eastern India with young leaves of the invasive plant WH collected from freshwater ponds on milk production, cow health, and its economic aspect. Eight cows aged between 3.7 to 6.7 years were considered for the study. In the control (0%) collection, they were fed with partial replacement feed composed of 5%, 10%, 15%, 20%, and 25% of total cow feed with young WH leaves. During the investigation, we assessed feed replacement-dependent changes in milk production and cow health to determine the EC₅₀, the optimal replacement level, and the efficacy of partial feed replacement with WH. From the study, it was known that an increase in WH% in the partially replaced feed had a positive effect on milk production while it had an adverse effect on health (stomach upset). Further, replacement feed with the range of 12.19-14.89% WH (maximum 15%) was identified to be optimum in terms of both milk production, cow health, and monetary contribution from milk output. So, the idea may be considered a step towards controlling the population of WH.

Keywords: Aquatic invasive plant, bio-utilization, cow health, milk production, toindex, TPR-FPR

INTRODUCTION

Eichhornia crassipes (Mart.) Solms, commonly known as Water Hyacinth (WH), is native to the Amazon basin (Eckert et al. 2016). Originally valued for its ornamental beauty, WH was introduced to many tropical and subtropical regions (Bakrim et al. 2022). However, its high proliferation rate and competitiveness have rated it among the top 10 most invasive weeds (Narayanan et al. 2017; Yan and Guo 2017), and it is included in the IUCN's list of the 100 most dangerous invasive species (Bakrim et al. 2022).

Introduced to India during the British period, WH has become a common nuisance in the country's freshwater bodies. The plant has broad, thick, glossy, ovate leaves, long, spongy stalks, and blackish-purple roots, sometimes rising to one meter above water (Heuzé et al. 2015). Its seeds remain viable for up to 28 years (Sullivan and Wood 2012), and its runners can double its population in just two weeks (Gettys et al. 2014; Punitha et al. 2015; Rezanía et al. 2015). This plant causes navigation interference, obstruction to water flow, evapotranspiration, and poses risks to hydroelectric and irrigation systems (Gettys et al. 2014; Dersseh et al. 2019). It adversely affects water quality, reducing dissolved oxygen, nutrient level, and pH (Gopal 1987; Rezanía et al. 2015), leading to drastic

changes in freshwater ecosystems and fish mortality and impacting fisheries (Datta et al. 2021). Infested areas face significant environmental challenges and socio-economic loss (Rezanía et al. 2015; Basaula et al. 2021).

Controlling WH is a global challenge. Common methods include chemical control (non-ecofriendly herbicides), physical control (non-cost-effective mowers and dredgers), and biological control (specific insects and fungi), with each method having their own merits and constraints (Greenfield et al. 2006). Reducing nutrient runoff from catchment areas can also help control WH (Pullen et al. 2014; Dersseh et al. 2019). While these methods aim to destroy WH in the populations, utilization offers another solution. Water hyacinth can be converted into enriched vermicompost (Sridevi et al. 2016) and used as animal feed (Tham 2015). However, eradicating WH is difficult, and most efforts focus on minimizing economic costs and ecological damage (Villamagna and Murphy 2010). Studies suggest that current WH management practices have not been fully successful in long-term control (Rezanía et al. 2015).

Water hyacinth is known to accumulate heavy metals, but young leaves collected from freshwater ponds are reported to have negligible heavy metal content (As 2.58 ppm, Pb 2.976 ppm, Zn 31.15 ppm, Cu 9.08 ppm, Cr 2.19

ppm) (Chaudhuri et al. 2008), and at least 18% crude protein making them suitable as fodder (Nyman et al. 2015; Indulekha et al. 2019). Young leaves have been utilized as a partial replacement for para-grass (*Brachiaria mutica* (Forssk.) Stapf) in ruminant diets (Tham 2012; Wimalaratne 2019). Tham (2015) used various forms of WH (fresh, wilted, pelleted, boiled, chopped, ground) as partial replacement in diets for ruminants, pigs, rabbits, fish, ducks, and geese with consistently positive results, reassuring the effectiveness of this method. WH also has high cellulose (36.5±0.9) and hemicellulose (22±0.5) levels, serving as energy sources for ruminants (Oyeoka et al. 2021). Therefore, to avoid bloat, studies recommend not exceeding 30% WH replacement for growing cattle (Greenfield et al. 2006) and 50% for sheep (Ilo et al. 2020).

The author's group has studied the plant diversity of freshwater lakes, estuaries, as well as coasts, along with their economic contributions (Manna et al. 2019; Roy et al. 2016; 2022). The present work aims to identify the potential of young WH leaves as a partial feed replacement for domestic lactating cows, calculate the optimum level of WH partial replacement, and assess its role in milk production and animal health. Additionally, the study discusses strategies for bio-utilization and WH population control in freshwater ponds of West Bengal, India, which involves replacing traditional feed with the WH partial replacement methods.

MATERIALS AND METHODS

Study region and selection of cows

The study was conducted in the fall of 2020 in the tropical coastal villages of Kotbarh (21.966556°N,

87.526306°E; rural area), Sarada (21.798556°N, 87.760306°E; rural area) and Kanthi (21.791333°N, 87.763944°E; semi-rural area), located in the East Medinipur District of West Bengal, India. These areas primarily derive their income from agriculture and animal husbandry. The study selected eight cows of a common indigenous breed, each in mid-lactation, and the cows were aged between 3.7 and 6.7 years, each accompanied by one calf aged between 7 and 9 months.

Preparation of fodder

In the control set, the cows were fed the traditional fodder used in the plains of West Bengal, India. This diet included paddy straw, starchy decoction derived from boiling rice, vegetable wastage, rice polish, and green grasses. For the experimental set, we introduced a unique feeding method. Fresh, young, unfurling leaves of the WH with minimally developed swollen petioles (Burke et al. 2014) were collected from the local wetlands situated 500 m to 1.5 km from nearby households. The collected leaves were manually chopped into small pieces (Mani 2018) and were boiled thoroughly for 20-25 minutes in unleaded earthen pots, with edible salt (NaCl) added according to the cow's requirements. After boiling, the broth was discarded (Masifwa et al. 2001), and freshwater was added. The diet was then carefully adjusted, ensuring that 5% to 25% of the total feed was replaced with boiled young leaves of WH (Table 1). Additionally, 3 to 5 L of starchy decoction derived from boiling rice (Bhaat'er Fan; Bhaat=Boiled Rice, Fan=sieved off starchy decoction in Bengali) were collected from households and added to the mixture. No commercial cow feed was used for either the control or experimental set.

Table 1. The feed chart for control (0% WH) and experiment (5-25% WH) sets for eight cows, each receiving 5 L of water daily

Set of cows	% and corresponding weight (g) of WH replacement in the feed	Rice polish (g)	Paddy straw (g)	Vegetable waste (g)	Total fodder (g)
Cow 1 and 2	0% (Control)	350	5,500	1,750	7,600
	5%; 380 g	332.5	5,225	1,662.5	
	10%; 760 g	315	4,950	1,575	
	15%; 1140 g	297.5	4,675	1,487.5	
	20%; 1520 g	280	4,400	1,400	
	25%; 1900 g	262.5	4,125	1,312.5	
Cow 3 and 8	0% (Control)	500	5,000	1,750	7,250
	5%; 362.5 g	475	4,750	1,662.5	
	10%; 725 g	450	4,500	1,575	
	15%; 1087.5 g	425	4,250	1,487.5	
	20%; 1450 g	400	4,000	1,400	
	25%; 1812.5 g	375	3,750	1,312.5	
Cow 4 and 5	0% (Control)	667	6,800	1,750	9,217
	5%; 460.85 g	633.65	6,460	1,662.5	
	10%; 921.7 g	600.3	6,120	1,575	
	15%; 1382.55 g	566.95	5,780	1,487.5	
	20%; 1843.4 g	533.6	5,440	1,400	
	25%; 2304.25 g	500.25	5,100	1,312.5	
Cow 6 and 7	0% (Control)	250	7,000	1,750	9,000
	5%; 450 g	237.5	6,650	1,662.5	
	10%; 900 g	225	6,300	1,575	
	15%; 1350 g	212.5	5,950	1,487.5	
	20%; 1800 g	200	5,600	1,400	
	25%; 2250 g	187.5	5,250	1,312.5	

Control readings for milk production and cow health were recorded for three consecutive days. Standard milking techniques were consistently applied throughout the study, with cows milked twice daily at fixed times each morning and evening to maintain uniformity in data collection. From the 4th day to the 10th day, 5% of each cow's total feed was replaced with boiled young leaves of WH, and milk production readings were taken from both morning and evening milkings on the 8th to 10th days. On the 11th day, the feed replacement percentage was increased to 10%, with production readings again recorded during both morning and evening sessions on the 15th to 17th days. This incremental replacement continued, reaching up to 25% by the 22nd day. Final milk production readings were collected from the morning and evening milkings on the 36th to 38th day. The detailed feed chart is presented in Table 1. Standard weighing machines were used to measure both traditional feeds and young leaves of WH, while standard measuring cylinders were employed for precise milk production measurements.

Statistical analysis

Results were analyzed using Anova: Two-Factor without replication in MS Excel, and percentage-wise results for WH were graphically plotted using a box plot in OriginPro 2018. The analysis focused on three main aspects: (i) Determining the optimum WH percentage by calculating the True Positive Rate (TPR) and False Positive Rate (FPR) for both productivity and health effects and deriving the trend line intersection; (ii) Calculating the optimum WH percentage using tolerance index, with calculations performed in R (ver 4.0.3) using *tolindex* package (Ver.0.1.0) (Pradhan and Guchhait 2019); and (iii) Calculating EC₅₀, which represents the concentration at which the replacement feed exerts half of its maximal 'complementary' effect, and identifying the optimal percentage by finding the crossover point where the complementation trend line intersects the health trend line.

The literature review suggested that incorporating WH into animal feed could increase milk production (production factor) (Sayanthan et al. 2024). However, our research has revealed the potential for feed replacement-dependent negative health impacts due to changes in traditional feed diets (health factor) (Abdelhamid and Gabr 1991; Tham 2012; Wimalarathne 2019; Oyeoka et al. 2021). These findings have significant implications for the animal nutrition and veterinary science field. The Receiver Operating Characteristic (ROC) analysis, a popular tool for clinical dose identification, was employed to predict efficacy from a given drug concentration and to identify optimal cut-off for a test, combining the highest true positive rate with the lowest false positive rate (Clements et al. 2018). To determine the appropriate cut-off for both production and health factors, proportions of Sensitivity/ True Positive Rate (TPR) and Specificity/ False Positive Rate (FPR) were plotted in an efficiency plot following Clements et al. (2018). TPR and FPR for WH concentrations ranging from 5% to 25% were calculated in MS Excel with the following formula:

$$\text{TPR (Production Factor) } 5\sim 25 / = \text{SUM(INC } 5\sim 25 : \text{INC } 25) / \text{SUM(INC } 5 : \text{INC } 25)$$

$$\text{FPR (Production Factor) } 5\sim 25 / = \text{SUM(DEC } 5\sim 25 : \text{DEC } 25) / \text{SUM(DEC } 5 : \text{DEC } 25)$$

$$\text{TPR (Health Factor) } 5\sim 25 / = \text{SUM(unhltly } 5\sim 25 : \text{unhltly } 25) / \text{SUM(unhltly } 5 : \text{unhltly } 25)$$

$$\text{FPR (Health factor) } 5\sim 25 / = \text{SUM(hlty } 5\sim 25 : \text{hlty } 25) / \text{SUM(hlty } 5 : \text{hlty } 25)$$

Where:

Inc: Number of cases with an increase in milk production

dec: Number of cases with a decrease in milk production

unhltly: Number of cases with unhealthy cows

hlty: Number of cases with healthy cows

The tolerance index for individual replacement feed concentrations ranging from 5% to 25% was calculated to determine the ideal feed tolerance (Clements et al. 2018). These calculations were performed in the R Program, identifying the highest TAVg as the ideal % of feed replacement. For this purpose, individual WH% replacement sets were assessed using two tolerance indices (T1 and T2) with the following formulas:

$$T_1 = ((C_{\text{step}} \times (1/D_{\text{step}} + H_{\text{step}})) / ((I_{\text{step}} + N_{\text{step}}) \times 1000)) \times I_{\text{step}}$$

$$T_2 = ((C_{\text{direct}} \times (1/D_{\text{direct}} + H_{\text{direct}})) / ((I_{\text{direct}} + N_{\text{direct}}) \times 1000)) \times I_{\text{direct}}$$

Where:

C_{step}: Stepwise sum of increase or decrease in milk produced by eight cows e.g., cumulative WH5% milk output of all cows (increase and decrease) compared to cumulative control output and henceforth

C_{direct}: Comparison of the sum of increase or decrease in milk produced by eight cows in various treatments (5% to 25%) directly with control output (0%)

D_{step}: Proportion of the decrease in milk production at a particular concentration evident from C_{step}

D_{direct}: Proportion of decrease in milk production at a particular concentration evident from C_{direct}

H: Proportion of cows without stomach upset at a particular concentration

I_{step}: Proportion of the increase in milk production at a particular concentration evident from C_{step}

I_{direct}: Proportion of the increase in milk production at a particular concentration evident from C_{direct}

N_{step}: Proportion of neutral/ no change in milk production at a particular concentration evident from C_{step}

N_{direct}: Proportion of neutral/ no change in milk production at a particular concentration evident from C_{direct}.

The EC₅₀ was calculated from the plot of the concentration-wise ratio of Effect (E)/Concentration (C) % (or % response) on the y-axis plotted against concentration in the x-axis, using a polynomial trend line of order 2. The EC₅₀ was determined as the value of x when y = 50. For calculating higher ECs, such as EC₇₅, Hill Slope or slope factor (H) was calculated using the formula: (Highest E/C – Lowest E/C)/(Highest Effect – Lowest Effect), which

measures steepness (higher H) or shallowness (lower H) of a curve, and standard feed replacement-response curve having H value of 1. The higher targeted EC, such as EC₇₅ (75% response), was then calculated from the formula $(75/(100-75))^{1/\text{value of H} \times \text{value of EC}_{50}}$.

For economic analysis, the rate of July 2020 for unskilled labor (Rs 257 for 8 hours, 30 minutes of work including 30 minutes of rest, but without additional food cost) was derived from the official website of the Labour Department, Government of West Bengal (WBLC 2020). During 2019–2020, the rate of milk/liter in rural West Bengal fluctuated between Indian Rupee (INR) 40–42; for the current analysis, the higher rate was considered. Polynomial equations of the trend line were solved for $y=50$ (EC₅₀) using the equation-solving platform of WolframAlpha (WolframAlpha 2020). No cost of fuel costs was additionally incorporated as farmers utilized plant debris from around the farmyard and waste rice straw to fuel the fodder preparation process.

RESULTS AND DISCUSSION

The study demonstrated that gradually replacing traditional feed with WH led to a significant increase in milk production ($F 73.16 > F$ critical 2.29, $P \leq 0.05$). Post hoc testing using the Holm-Bonferroni correction further clarified these findings. Comparisons showed significant increases in milk production at 5%, 10%, and 15% WH feed replacement levels (corrected P-values of 0.0083, 0.0042, and 0.0032, respectively). In comparison, no significant changes were observed at 20% and 25% replacement levels (corrected P-values of 0.1674 and 0.8587, respectively). However, when feed replacement reached 20%, one out of eight cows showed signs of stomach upset, indicated by irregular excreta and decreased milk productivity, which necessitated a return to the control feed level for that cow. At 25% replacement, six out of eight cows experienced stomach upset, and both the mean and median milk output values showed a noticeable drop post-15% WH replacement (Figure 1). These findings align with Tham (2012), who also observed signs of indigestion in cattle after 25% feed replacement. Due to these adverse effects, feed replacement with a higher concentration of *E. crassipes* was halted, and cows were returned to their normal (control) feed regime. It's important to note that milk output responses varied among individual cows, with ANOVA indicating a significant difference in feed utilization capacities ($F=4.86 > F$ critical 2.49, $P \leq 0.05$). This highlights the need for personalized feeding strategies to ensure the well-being of each cow.

The optimum feed replacement level with TPR-FPR analysis

The True Positive Rate (TPR) and False Positive Rate (FPR) for WH % feed replacement, along with their gradient proportions, are presented in Tables 2 and 3. The alternative hypothesis posited that increasing WH% would positively affect productivity but negatively affect health. For productivity, true positives were recorded up to 15%

WH. Beyond 15% WH (20–25%), false negative results were observed. Conversely, false positive results for productivity were noted within the 5–15% WH concentration, while true negative cases were observed in the 20–25% WH range.

For health, true positives were recorded in the 20–25% WH range, while false negative cases were noted within the 5–15% WH range. Similarly, false positive results for productivity were found within 5–15% WH concentration, and true negative cases were noted in 20–25% WH range. The accuracy of the TPR-FPR analysis for WH productivity was 0.813, compared to 0.4 for the same analysis for health. Based upon the x-axis values at the intersection of individual TPR and FPR trend lines for productivity and health (Figures 2 and 3), the ideal WH% for replacement feed was determined to be between 12.192–14.765% (Table 4).

The optimum feed replacement level with toindex

The toindex, which incorporates productivity, health factors, and neutral values (where effects do not change between treatment percentages), was calculated for each WH replacement percentage. In Table 5, for immediate treatment comparison (T1), WH 10% showed the highest value of 4.60, whereas the control-based comparisons (T2) showed the highest value of 11.95 for WH 15%. On average, WH 15% had the highest Tavg, followed by WH 10%. However, despite WH 15% achieving the highest T2 value, its T1 value (3.60) was lower than the T1 (3.75), suggesting WH 10% as a more consistent option with high T1 (4.60) and T2 (8.35) values. Notably, the Tavg values for WH 20% and 25% were negative, indicating a synergistic decline in health and milk production. The toindex was developed as an R package and hosted on GitHub (Pradhan and Guchhait 2019).

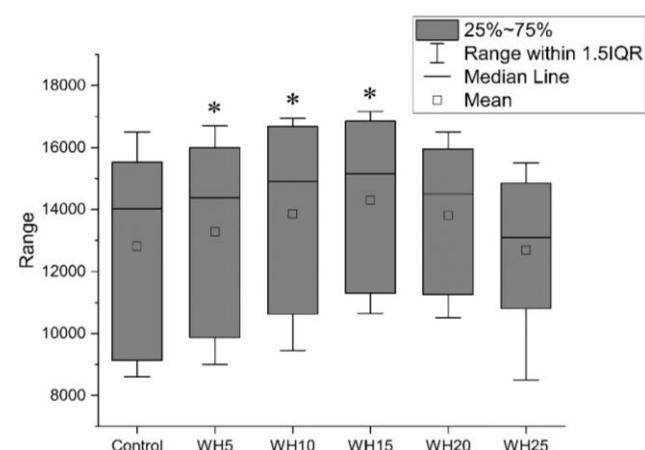


Figure 1. Cumulative quantity (mL) of milk produced by eight cows/day across various experimental stages. Boxes represent the first quartile (25%) to the third quartile (75%) of data, whiskers indicate the range within the $1.5 \times$ Interquartile Range (IQR), a bar within each box represents the median, and a small square represents the mean. An asterisk (*) above the bars for WH5, WH10, and WH15 denotes statistically significant increases in milk production compared to the control (Holm-Bonferroni adjusted $p < 0.05$)

Table 2. TPR-FPR analysis of productivity related to WH partial replacement

WH%	Decrease	Increase	TPR	FPR	TPR proportion	FPR proportion
5		16	1	1	0.389	0.218
10		16	0.746	1	0.290	0.218
15		16	0.492	1	0.191	0.218
20	7	9	0.238	1	0.0926	0.218
25	10	6	0.095	0.588	0.0370	0.128

Note: TPR: True Positive Rate, FPR: False Positive Rate, Decrease: Decrease in milk production, Increase: Increase in milk production. Accuracy of TPR-FPR analysis: 0.813

Table 3. TPR-FPR analysis of cow health related to WH partial replacement

WH%	Healthy	Unhealthy	TPR	FPR	TPR proportion	FPR proportion
5	8		1	1	0.206	0.384
10	8		1	0.758	0.206	0.290
15	8		1	0.515	0.206	0.198
20	7	1	1	0.273	0.206	0.105
25	2	6	0.857	0.061	0.176	0.023

Note: TPR: True Positive Rate, FPR: False Positive Rate, Healthy: Number of healthy cows, Unhealthy: Number of unhealthy cows. Accuracy of TPR-FPR analysis: 0.4

Table 4. Trendline equations of TPR-FPR for productivity and health and value of x-axis at the intersection of individual TPR and FPR trendlines of productivity and health

Proportion	Trendline equation	R ²	Intersection
FPR (productivity)	$y = -0.0005x^2 + 0.0118x + 0.1641$	0.8571	12.192
TPR (productivity)	$y = 0.0002x^2 - 0.0254x + 0.5136$	0.9974	
FPR (Health)	$y = 7E-05x^2 - 0.0201x + 0.4837$	0.9998	14.765
TPR (Health)	$y = -0.0002x^2 + 0.0039x + 0.1882$	0.8571	

Note: Trendline equation: Quadratic equation of order 2

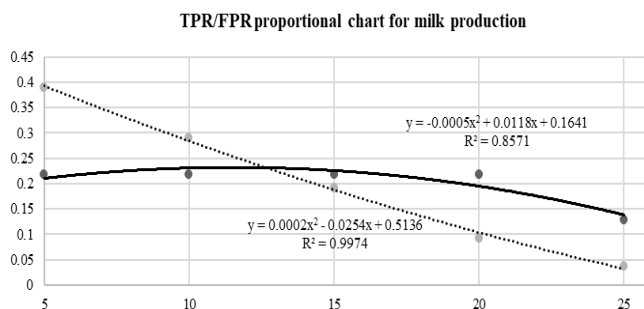


Figure 2. TPR/FPR proportional chart for milk production

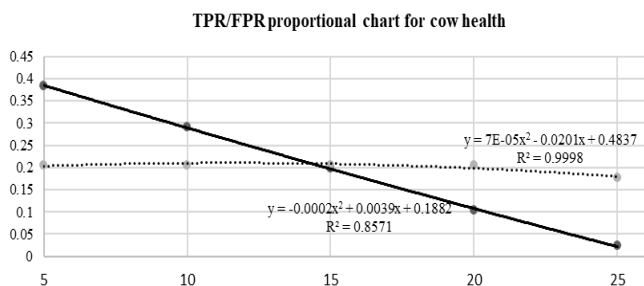


Figure 3. TPR/FPR proportional chart for cow health

Calculation of EC₅₀

EC₅₀ values were calculated for both productivity across seven cows (with WH administration ranging from 0-25%) and for cow number 5 (with WH administration ranging from 0-20%), as well as for health. The EC₅₀ values for productivity ranged from 5.28 to 6.57, while for health, the EC₅₀ value was 5.19 (Table 6). This indicated that a 5% WH replacement was nearly equivalent to 50% of the effective concentration for the cows. Additionally, the Hill Slope (H) value for the health factor was 0.253, suggesting less accuracy in predicting higher EC values (e.g., EC₇₅, EC₉₀). In contrast, the H value for productivity was close to 1, indicating the productivity curve was closer to the standard curve, which allowed for a more accurate prediction of higher EC values based on productivity.

Economic analysis

In rural southern parts of West Bengal, it was found that 73.63 minutes were invested daily per cow for tasks ranging from cleaning the cows and cowshed to preparing feed and milking. Collecting young WH leaves was not a dedicated task due to the abundance of waterbodies and available WH vegetation around the villages. This activity was typically carried out by locals on the way home from other prioritized tasks, taking between 4.69 to 21.16 minutes and resulting in the collection of 0.36 to 2.3 kg of young WH leaves (Tables 1 and 7). The WH leaves

collection times were added to the cow rearing and milking 'control' time, however calculations were also made excluding WH harvesting time. Including the WH collection time led to a gradual increase in investment costs, while excluding collection time showed a decline in costs supplemented by WH input, which refers to the use of WH leaves as a cost-effective feed supplement.

WH at 15% had the highest average monetary value (INR) of milk per day, yielding a higher return and profit compared to the control when calculated without a time

factor. This finding underscores the potential for profit at WH 15%, which should be a source of optimism for farmers and policymakers. However, when factoring in the collection time, the control showed the highest profit margin (INR 27.65), with WH 10% close behind (INR 27.14). It was also observed that investing additional time beyond WH 15% did not yield adequate returns in milk production and resultant monetary benefits. Therefore, if WH harvesting time was not considered, collectors could still achieve profit at WH 15% (Table 7).

Table 5. Optimal feed replacement levels with Tolindex values for milk productivity and bovine health: Comparative analysis across WH percentages

	5%	10%	15%	20%	25%	Trend line equation	Max X
T1	3.75	4.60	3.60	-8.00	-5.09	$y = 0.0007x^4 - 0.0441x^3 + 0.8641x^2 - 5.6424x + 15.431$	14.89
T2	3.75	8.35	11.95	7.79	-0.38	$y = 0.0023x^4 - 0.1245x^3 + 2.2884x^2 - 16.593x + 43.664$	12.99
T _{Avg}	3.75	6.48	7.78	-0.11	-2.73	$y = 0.0015x^4 - 0.0843x^3 + 1.5763x^2 - 11.118x + 29.548$	13.79

Note: T1: Compared to immediate set, T2: Compared to control, Avg: Average of T1 and T2, R2: 1 for all three equations, Trend line equation: Quadratic equation of order 4, Max X: Value of X axis on the peak of trend line

Table 6. Trend line equations derived from WH% concentration vs average effect/concentration plot. EC₅₀ was derived from the x value of the trend line when y=50

	Trend line equation	R ²	EC ₅₀	Hill Slope (H)
Cow 5	$y = -0.1405x^2 + 6.9411x + 17.286$	0.9999	5.28	0.987
Cows 1,2,3, 4,6,7,8	$y = -0.0908x^2 + 5.5587x + 17.408$	0.9992	6.57	1.27
Health	$y = -20.395x^2 + 120.84x - 27.373$	0.9938	5.19	0.253

Note: Trend line equation: Quadratic equation of order 2. Values of Cow 5 and the other 7 cows were taken separately

Table 7. Role of WH and time investment for its collection in daily profit from the sale of milk

WH%	Milk production (mL/day)	Value (INR 42/L)	Return above control (INR)	Time investment (INR)	With time factor			Without time factor		
					Feed investment (INR)	Investment compared with control (INR)	Profit (INR)	Feed Investment (INR)	Investment compared with control (INR)	Profit (INR)
0	2,134.38	89.64	-	73.63	61.99	-	27.65	22.57	-	67.07
5	2,212.50	92.93	+3.28	84.18	66.68	+4.69	26.24	21.61	-0.96	71.32
10	2,308.33	96.95	+7.31	91.81	69.81	+7.82	27.14	20.65	-1.92	76.30
15	2,383.33	100.10	+10.46	99.94	73.20	+11.21	26.90	19.69	-2.88	80.41
20	2,235.71	93.90	+4.26	110.50	77.88	+15.89	16.02	18.72	-3.85	75.18
25	2,225.00	93.45	+3.81	119.44	83.15	+21.16	10.30	19.20	-3.37	74.25

Note: Milk production: Average quantity of milk (mL)/day, Value: Average monetary value (INR) of milk/day, Return: Increase in milk production above control in monetary terms, Time investment: Average time invested (minutes) per cow, Feed investment: Investment on rice polish, straw, salt

Environmental considerations

Unmanaged WH populations in water bodies are known to outcompete native aquatic species, reduce oxygen levels for aquatic wildlife, and create ideal habitats for water-borne vectors like mosquitoes (Lahon et al. 2023). Daily harvesting of WH cover would control the spread of WH colonies and allow sunlight to reach native submerged plants. These plants, in turn, would help restore the oxygen-depleted by fast-growing WH colonies, thus supporting aquatic wildlife survival.

The study showed that an optimal 15% level of partial replacement feed would result in each cow utilizing 1.24 kg

of WH per day, while a 10% level would amount to 0.83 kg per day (Table 1). The bovine density in the East Medinipur District is reported to be 220/km² (NDDB 2017), suggesting that within a 1 km² area, 182.6 kg to 277.76 kg of WH could be utilized daily. This could be a significant step towards controlling WH populations.

Lahon et al. (2023) reported that WH biomass output is highest during the summer season (43-51%; May-August), drops to 3-11% during September-December, and is 16-31% during January-February. Therefore, harvesting efforts to eradicate WH should be intensified during September-February when the plants are already stressed

due to low biomass output. This strategy could potentially lead to significant economic benefits. However, maximum harvesting from an economic perspective may be achieved during May-August.

The use of WH as animal feed is prevalent in Southeast Asian countries, both for ruminants and non-ruminants, in both raw and boiled forms (Masifwa et al. 2001; Tham 2015). However, few studies have tested bovine feed tolerance to WH replacement (Tham 2015; Ilo et al. 2020). The current study suggests an optimal WH replacement level in a feed to be 12.19-14.89% (maximum 15%), which is within the previously suggested maximum of 30% for cattle (Ilo et al. 2020). The milk output from the optimal WH replacement of 15% was found to be 2.3833 L; however, the lack of comparative data from other studies underscores the need for further research in this area.

Regarding heavy metal content, WH leaves collected from freshwater ponds have been reported to contain 2.976 ppm of Pb, which is below the level found in paragrass (5.84 ppm) and much below the alfalfa haylage (271 ppm). In the Indian context, WH has the lowest Zn content (31.15 ppm) compared to hybrid napier (43.426 ppm). However, only in the case of As and Cu do sorghum (0.424 ppm and 8.685 ppm, respectively) have lower values than WH (2.58 ppm and 9.08 ppm) (Jafari 2010; Nyman et al. 2015). Compared to bovine feeds discussed in the study of Jafari (2010), leaves of WH from fresher ponds are reported to have much lower amounts of heavy metal content (Nyman et al. 2015). However, it is recommended to avoid WH leaves collected from polluted areas, sewage canals (Nyman et al. 2015), as well as Arsenic and Fluoride affected areas (Chaudhuri et al. 2008).

In conclusion, the experiment proved to be an efficient means of partially replacing traditional feed for dairy cattle in eastern India while managing the population of WH. Analyses such as TPR-FPR for calculating the optimum WH% and the development and use of the toindex R package yielded comparable results, with ideal WH levels ranging from 12.19-14.77% (TPR-FPR) and 12.99-14.89% (tolindex). While a 5.19-6.57% WH replacement initiated a 50% response in milk production (EC₅₀), the optimal effect was observed within 12.19-14.89% WH, with a maximum effect at 15%. At this level, milk production and associated monetary returns were maximized, resulting in an 8.15-11.67% increase in milk yield and additional returns of INR 7.31-10.46 over control values when WH was used as a partial replacement feed replacement within the 10-15% range. While heavy metal accumulation in WH is a known concern, studies have reported low heavy metal content in young WH leaves. Therefore, to minimize this risk, the study recommends selecting pollution-free freshwater sites, collecting only young leaves, and boiling them before use. Future research could extend this study by evaluating the protein, fat, and heavy metal composition of milk derived from WH-fed cows to assess both nutritional benefits and safety better. This additional data would contribute valuable insights to support the broader application of WH as a viable partial feed replacement.

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