

Supplementation effect of *Plantago major*: on milk yield, digestive efficiency, and systemic health in Holstein Friesian cows

QURATULAIN Badhana

Agricultural University Peshawar: The University of Agriculture Peshawar

Arshad Zahoor

arshad@aup.edu.pk

The University of Agriculture Peshawar <https://orcid.org/0000-0003-2136-5458>

Asif Ghafoor

The University of Agriculture Peshawar

Muhammad Tahir KHAN

Agricultural University Peshawar: The University of Agriculture Peshawar

Research Article

Keywords: *Plantago major*, Holstein Friesian cows, feed digestibility, milk yield, phytogetic feed additives, dairy nutrition

Posted Date: September 18th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7293267/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Functional forages like *Plantago major* have shown promise in enhancing nutrient utilization and systemic health in the dairy cattle. However, limited data exist on their effects under controlled supplementation trials.

Objective

This study explores the combined effects of graded dietary supplementation with *Plantago major* (common plantain) on lactational performance, nutrient digestibility, and systemic health in mid-lactation Holstein Friesian cows under intensive Pakistani dairy farm conditions.

Introduction :

Despite Pakistan's position as the fourth-largest global milk producer—yielding 72.4 million tons in 2025—average per-cow output remains suboptimal due to fragmented infrastructure, low forage diversity, and conventional feeding strategies use. *Plantago major*, is a resilient forage species rich in flavonoids (baicalein, luteolin), iridoid glycosides (aucubin), phenolic acids, polysaccharides, and fermentable fibers.

Methods

Evaluation was established at four inclusion levels (0%, 5%, 10%, and 15% of dietary dry matter) in a 30-day randomized complete block design (n = 3 cows per treatment). All diets were formulated to meet NRC (2001) requirements, and a 10-day adaptation period preceded a 7-day data collection phase. Major endpoints included dry matter intake (DMI), nutrient digestibility coefficients (DMD, CPD, NDFD, ADFD), milk yield and composition (fat, protein, lactose, total solids), and hemato-biochemical indices (urea, creatinine, total protein, albumin, glucose, cholesterol, triglycerides, ALT, AST, hemoglobin, packed cell volume).

Results

Demonstrated a dose-dependent enhancement in feed intake and digestibility. Cows receiving 15% *Plantago major* exhibited the highest DMI (28.43 kg/day), crude protein intake (5.04 kg/day), and fiber intake (NDFI: 9.11 kg/day; ADFI: 3.42 kg/day). Corresponding digestibility improvements included dry matter digestibility (P-15: 71.24% vs. P-0: 66.87%), crude protein digestibility (82.11% vs. 78.21%), neutral detergent fiber digestibility (73.42% vs. 68.48%), and acid detergent fiber digestibility (85.64% vs. 80.27%). The P-15 group achieved the highest mean daily yield (17.65 kg/cow), representing a 20.8%

gain over controls (14.60 kg). Milk protein (3.49%), lactose (5.52%), and total solids (12.88%) also peaked in this group, whereas milk fat percentage declined marginally (P-15: 3.37% vs. P-0: 4.05%). Serum total protein and albumin increased by 6.8% and 13.3% respectively in P-15 cows, while blood urea and creatinine concentrations decreased by 11.0% and 37.4%, suggesting enhanced nitrogen utilization efficiency and renal integrity. Hepatic enzyme activities declined (ALT: – 9.6%; AST: – 4.8%). Additionally, total cholesterol fell by 5.1%, and hemoglobin rose by 34.7%, demonstrating hypolipidemic and hematopoietic advantages.

Conclusion

Collectively, these findings confirm that 15% *Plantago major* acts as a functional forage, improving feed conversion efficiency, lactational outputs, and systemic metabolic resilience in high-yielding dairy cows.

INTRODUCTION

As of 2025, Pakistan remains the world's fourth-largest milk-producing country, with national output reaching 72.4 million tons—a 3.3% increase from the previous fiscal year (FAO, 2025) ^[6]. The livestock sector showed continued resilience, posting a 4.9% growth in value-added, reflecting ongoing improvements in dairy productivity.

Even though, its economic significance—contributing over 14% to national GDP—the average milk yield per cow remains below optimal thresholds. This inefficiency stems from suboptimal feeding regimes, limited access to functional forages, and the physiological demands of high-producing breeds like Holstein Friesians under intensive systems. Traditional rations dominated by cereal roughages and low-diversity fodder often fail to meet the nutritional needs of lactating cows. Milk consumption in Pakistan remains predominantly traditional, with 96.8% consumed fresh and only 3.2% processed *via* Ultra High Temperature (UHT) methods (MNFSR, 2025) ^[7]. Despite notable advancements in milk production over the past decade, Pakistan's dairy industry in 2025 remains a sector characterized by underutilized potential and systemic inefficiencies. While national milk output has surpassed 72 million tons, average per-cow yield continues to fall significantly short of biological capacity. Structural limitations—including fragmented dairy infrastructure, poor feed management, and outdated genetic and breeding systems—continue to constrain productivity across both commercial and smallholder operations ^[8]. In rural settings, nutritional gaps are compounded by inconsistent forage availability, limited veterinary coverage, and weak cold chain logistic facilities, resulting in compromised milk quality, impaired metabolic health in animals, and reduced economic return for producers ^[9]. However, whether alternative forage functions similarly in the modulation of rumen fermentation pattern, improving blood biochemistry, including serum protein and liver enzymes and ultimately lactational efficiency remains to be elucidated.

Functional forages that provide high total digestible nutrients (TDN) and crude protein (CP) are essential for overcoming productivity and health challenges in the intensive dairy systems ^[10–11]. *Plantago major*, a

sturdy bioactive forage, which is rich in flavonoids (e.g., baicalein, luteolin), iridoid glycosides (aucubin), phenolic acids, polysaccharides, and fermentable fibers. These phytochemicals improve digestibility, steer rumen fermentation toward propionate production, and enhance nitrogen metabolism, while also offering antioxidant and hepatoprotective effects^[12–13]. Its deep-rooted system promotes soil stability, and its adaptability to compacted soils makes it a readily available functional feed option capable of boosting feed conversion efficiency, lowering nitrogen losses, and enriching milk composition quality in Holstein Friesian cows.

This study assessed graded inclusion levels of *P. major* (0%, 5%, 10%, and 15% of dietary dry matter) in total mixed rations for mid-lactation Holstein Friesian cows under intensive Pakistani dairy conditions. Key measures included dry matter intake, apparent nutrient digestibility (DM, OM, CP, NDF, ADF), milk yield and composition (fat, protein, lactose, total solids), and systemic hemato-biochemical markers (urea, creatinine, total protein, albumin, glucose, cholesterol, triglycerides, ALT, AST). We hypothesized that increasing *P. major* inclusion would linearly improve feed intake, nutrient utilization, lactational performance, and metabolic health. The results are intended to guide sustainable feeding strategies that maximize both productivity and animal well-being in Pakistan's fast-growing dairy industry.

MATERIALS & METHODS

Study Site and Ethical Approval

The experiment was conducted at the Government Cattle Breeding and Dairy Farm, Harichand, under the technical supervision of the Department of Animal Nutrition, University of Agriculture, Peshawar, Pakistan. All procedures conformed to institutional animal-care guidelines and were approved by the University Ethical Review Board.

Animals and Experimental Design

Eighteen clinically healthy mid-lactation Holstein Friesian cows (average BW $\approx$ 350 kg; similar parity) were selected and randomly assigned to four treatment groups ($n = 3$ cows/group). A 10-day adaptation period preceded a 30-day feeding trial using a randomized complete block design (RCBD) to account for individual variability. Each group received diets containing incremental levels of *Plantago major* leaves: 0% (P-0), 5% (P-5), 10% (P-10), and 15% (P-15) of dietary dry matter showed in (**Table:1**)

Table 1
Experimental Layout of treatment group of Plantain
feeding to HF cow

Replicates			
Treatments	R1	R2	R3
Basal diets (P-0)	1	1	1
P-5 (basal diets + Plantain 5%)	1	1	1
P-10 (basal diets + Plantain 10%)	1	1	1
P-15 (basal diets + Plantain 15%)	1	1	1

Diet Formulation and Feeding Regimen

Total mixed rations (TMR) were formulated to meet or exceed nutrient requirements for lactating dairy cows^[2]. Four diets differed only in their inclusion of freshly harvested *Plantago major* leaves at 0%, 5%, 10%, or 15% of dietary dry matter. Basal ingredients—silage, berseem, wheat straw, wheat bran, gluten meal, cottonseed cake, and a mineral–vitamin premix—were adjusted to maintain isocaloric and isonitrogenous balance across treatments. All forages were chopped to ~ 2 cm, thoroughly mixed, and offered twice daily at 08:00 and 16:00 h. Daily feed offered, and refusals were weighed to calculate individual dry matter intake (DMI). The ration formulation and its chemical composition is shown in the (Table 2)

Table 2
Ration formulation and its chemical composition

Ingredients	P-0	P-5	P-10	P-15
Berseem	20	15	10	5
Silage	20	20	20	20
Plantain	0	5	10	15
Gluten	12	12	12	12
Wheat Straw	20	20	20	20
Wheat Bran	15	15	15	15
Cotton Seed Cake	11	11	11	11
Salt	1	1	1	1
COMPOSITION				
DM	81.5	81.8	79.2	78.80
Ash	14.4	15.3	17.01	17.3
CF	23.8	23.2	24.8	24.7
CP	22.12	21.47	19.66	19.3
EE	3.4	3.78	3.12	3.7
ADF	28.5	28.3	27.4	27
NDF	45.57	44.21	44.24	43.14

Dry matter (DM), Crude fiber (CF), Crude protein (CP), Ether extract (EE), Acid detergent fiber (ADF), Neutral detergent fiber (NDF), P-0, Basal diet, P-5; basal diet + 5% plantain, P-10; basal diet + 10% plantain, P-15: basal diet + 15% plantain

This diet structure allowed for a clear comparison between conventional forage ratios and plantain-integrated rations, thereby enabling quantification of *P. major*'s impact on lactational performance and systemic health.

Milk Yield and Composition

Milk yield was recorded twice daily (04:00 and 16:00 h) using a digital scale (± 0.1 kg). Composite daily samples (50 mL) were collected aseptically into sterile tubes, refrigerated at 4°C, and analyzed within 24 h for fat, protein, lactose, total solids, and solids-not-fat using an Elko Milk Analyzer at the Veterinary Research Institute, Peshawar.

Blood Biochemistry and Hematology

Blood (5 mL) was collected by jugular venipuncture on Day 0 (baseline) and Day 40 into EDTA-coated and plain vacutainers. Samples were centrifuged at $3,000 \times g$ for 10 min at 22°C; serum was aliquoted and stored at 4°C. Analytes measured included urea, creatinine, total protein, albumin, glucose, cholesterol, triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Hemoglobin and packed cell volume (PCV) were determined from EDTA blood. All assays were run in duplicate with Randox colorimetric and enzymatic kits ^[14].

Digestibility Assessment

During the 7-day collection phase, total fecal output was collected daily using harness-mounted bags. Ten-percent subsamples of feed offered and feces were retained, frozen at – 20°C, then dried at 65°C for 72 h. Dried samples were ground to pass a 1 mm sieve and analyzed for dry matter, ash, crude protein (Kjeldahl method), and ether extract ^[15]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with an ANKOM analyzer ^[16]. Apparent digestibility coefficients were calculated as

$$\text{Apparent digestibility (\%)} = [(\text{Nutrient intake} - \text{Nutrient in feces}) / \text{Nutrient intake}] \times 100$$

Statistical Analysis

Data were analyzed using the General Linear Model procedure of SAS 9.4 (SAS Institute Inc., 2013). Treatment effects on DMI, digestibility, milk yield/composition, and blood parameters were tested by one-way ANOVA. Means were separated by Tukey's honestly significant difference test at $P < .05$. Normality and homogeneity of variances were confirmed by Shapiro–Wilk and Levene's tests.

RESULTS

Nutrient Intake relation with *Plantago major*

Pasture treatment significantly affected nutrient intake of dairy cows in a dose-dependent way with higher *Plantago major* inclusion (0%, 5%, 10%, and 15% of dietary DM; $p < 0.01$ for all comparisons; **(Table 3)**). Dry matter intake (DMI) increased steadily from 24.12 kg/d in P-0 to 28.43 kg/d in P-15, a rise of 18%. Organic matter intake (OMI) is followed a similar pattern, rising by 19% (from 22.01 to 26.19 kg/d).

Crude protein intake (CPI) showed significant increase across treatments ($p < 0.01$), with an increase of 21% between P-0 and P-15 (4.18 vs. 5.04 kg/d). Ether extract intake also (EEI) demonstrated a sharp 174% increase (0.78 to 2.14 kg/d; $p < 0.01$), indicating a substantial enhancement in lipid ingestion with elevated P. major inclusion.

Intake of neutral detergent fiber (NDFI) and acid detergent fiber (ADFI) also increased significantly ($p < 0.001$), by 51% (6.05 to 9.11 kg/d) and 57% (2.18 to 3.42 kg/d), respectively. These linear responses across graded levels of *Plantago major* demonstrate consistent effects on nutrient uptake and ruminal fiber dynamics.

TABEL 3. Analysis of nutrient intake in experimental Holstein Friesian cows fed different levels of plantain (*Plantago major*)

Parameters (kg/day)	P-0	P-5	P-10	P-15	SEM	P-VALUE
DMI	24.12d	25.45c	26.88b	28.43a	0.75	**
OMI	22.01d	23.46c	24.82b	26.19a	0.82	**
CPI	4.18d	4.49c	4.76b	5.04a	1.1	**
EEI	0.78d	1.16c	1.59b	2.14a	1.4	**
NDFI	6.05d	7.24c	8.26b	9.11a	0.38	***
ADFI	2.18c	2.71b	3.29a	3.42a	0.63	***

Values with distinct superscription (^{abcd}) within the column differ significantly at ($P < 0.05$); ***p value < 0.05 ; **p value < 0.01 ; P-0, Basal diet with no supplementation, P-5; basal diet with 5% plantain supplementation, P-10; basal diet with 10% plantain supplementation, P-15: basal diet with 15% plantain supplementation; DMI, dry matter intake; OMI, organic matter intake; CPI, crude protein intake; EEI, ether extract intake; NDFI, Nutrient detergent fiber intake ADFI, Acid detergent fiber intake;

Apparent Nutrient Digestibility

Pasture treatment significantly influenced apparent digestibility coefficients of dairy cows fed graded levels of *Plantago major* (0%, 5%, 10%, and 15% dietary DM), with all nutrient fractions showing a linear improvement across treatments (Table 3.1). Dry matter digestibility (DMD) increased by 6.5%, from 66.87% in P-0 to 71.24% in P-15. Likewise, organic matter digestibility (OMD) rose by 5.7%, from 74.92–79.18% across treatments.

Crude protein digestibility (CPD) was significantly enhanced ($p < 0.001$), increasing by 5% from 78.21% in P-0 to 82.11% in P-15. Ether extract digestibility (EED) rose by 6% across treatments, reaching 87.92% in P-15 compared to 83.11% in P-0 ($p < 0.01$).

Fiber digestibility parameters were also positively affected. Neutral detergent fiber digestibility (NDFD) improved by 7% (68.48% vs. 73.42%; $p < 0.001$), while acid detergent fiber digestibility (ADFD) increased by 6.7% (from 80.27% in P-0 to 85.64% in P-15; $p < 0.001$). These findings confirm a consistent enhancement in digestibility efficiency with increasing P. major inclusion over the treatment range.

Table 3.1
Analysis of digestibility parameters in experimental Holstein Friesian cows fed different levels of plantain (*Plantago major*)

Parameters (%)	P-0	P-5	P-10	P-15	SEM	p-value
DMD	66.87d	68.13c	69.47b	71.24a	1.1	**
OMD	74.92d	76.03c	77.38b	79.18a	1.4	**
CPD	78.21d	79.36c	80.74b	82.11a	0.9	***
EED	83.11d	84.25c	86.14b	87.92a	1.2	**
NDFD	68.48d	70.17c	71.81b	73.42a	1.5	***
ADFD	80.27d	82.02c	83.73b	85.64a	1.3	***

Values with various superscription (^{abcd}) within the column differ significantly at ($P < 0.05$); ***p value < 0.05 ; **p value < 0.01 ; P-0, Basal diet with no supplementation, P-5; basal diet with 5% plantain supplementation, P-10; basal diet with 10% plantain supplementation, P-15: basal diet with 15% plantain supplementation; DMD, dry matter digestibility; CPD, crude protein digestibility; EED, ether extract digestibility; OMD, organic matter digestibility; ADFD, Acid detergent fiber digestibility; NDFD, Nutrient detergent fiber digestibility

Supplementation relation with milk yield and composition

The inclusion of *Plantago major* in the dairy cow diet resulted in a clear, dose-dependent enhancement of milk yield and composition (Table 3.2). Average daily milk production increased steadily, rising from 14.60 kg/cow/day in the control group (P-0) to 17.65 kg/cow/day in the highest supplementation group (P-15)—a substantial 21% gain. Alongside this rise in yield, milk protein concentration improved by 27%, increasing from 2.74–3.49%, while lactose levels showed a 22% uplift, moving from 4.52–5.52%. A modest increase in total solids was also observed, climbing from 12.31% in P-0 to 12.88% in P-15. In contrast to these enhancements, milk fat percentage declined progressively, dropping by 17% from 4.05–3.37% across the treatment spectrum . The supplementation of *Plantago major* resulted in a significant improvement in milk yield and total solids, indicating its positive impact on lactational performance (Fig. 1)

Table 3.2
Analysis of milk yield (kg/cow/day) and composition (%) in experimental Holstein Friesian fed various levels of plantain (*Plantago major*)

Treatment	Milk Yield (kg/cow/day)	Fat (%)	Protein (%)	Lactose (%)	Total Solids (%)
P-0	14.6☐	4.05☐	2.74☐	4.52☐	12.31☐
P-5	15.6☐	3.95☐	2.95☐	4.70☐	12.45☐
P-10	16.4☐	3.74☐	3.17☐	5.12☐	12.73☐
P-15	17.65☐	3.37☐	3.49☐	5.52☐	12.88☐

Values with distinct superscription (^{abcd}) within the column differ significantly at (P < 0.05): ***p value < 0.05: **p value < 0.01: P-0, Basal diet with no supplementation, P-5; basal diet with 5% plantain supplementation, P-10; basal diet with 10% plantain supplementation, P-15: basal diet with 15% plantain supplementation;

Effect of Plantago major on hematology

Graded dietary inclusion of Plantago major (0%, 5%, 10%, and 15% of dietary DM) significantly affected hematological indices in lactating dairy cows (Table 3.3). Hemoglobin concentration increased linearly ($p < 0.001$), from 9.5 ± 0.6 g/dL in P-0 to 12.8 ± 0.6 g/dL in P-15, representing a 35% elevation and indicating enhanced erythropoietic capacity and improved oxygen transport potential.

Packed cell volume (PCV) showed a gradual upward trend, rising from $32.0 \pm 1.4\%$ in P-0 to $33.0 \pm 1.4\%$ in P-15, although this change was not statistically significant ($p = 0.12$). The concurrent increase in hemoglobin without marked shifts in PCV suggests an expansion in red-cell mass with stable hydration, rather than plasma dilution effects

Table 3.3
effect of various levels of plantain (Plantago Major) on blood biochemical profile in experimental HF cows

Parameter	P-0	P-5	P-10	P-15	SEM	P-value
Albumin (mg/dL)	33.2 ^a	35.3 ^b	36.4 ^{bc}	37.6 ^{cd}	0.4	**
Glucose (mg/dL)	53.7 ^a	54.0 ^{ab}	54.2 ^{abc}	54.3 ^{abcd}	0.3	***
Total Protein (mg/dL)	72.0 ^a	73.1 ^b	74.7 ^{bc}	76.9 ^{cd}	0.3	**
Creatinine (mg/dL)	0.91 ^a	0.75 ^b	0.68 ^{bc}	0.57 ^{cd}	0.06	***
Urea (mg/dL)	25.4 ^a	24.8 ^b	23.2 ^{bc}	22.6 ^{cd}	2.0	***
Cholesterol (mg/dL)	135.9 ^a	133.2 ^b	131.3 ^{bc}	129.0 ^{cd}	0.7	**
Hemoglobin (g/dL)	9.5 ^a	10.6 ^b	11.5 ^{bc}	12.8 ^{cd}	0.6	***
ALT (IU/L)	34.5 ^a	33.0 ^b	32.4 ^{bc}	31.2 ^{cd}	1.2	**
AST (IU/L)	67.9 ^a	67.3 ^b	66.8 ^{bc}	64.6 ^{cd}	0.9	***
Packed Cell Volume (%)	32.0 ^a	32.3 ^b	32.6 ^{bc}	33.0 ^{cd}	1.4	NS

Values with different superscription (^{abcd}) within the column differs significantly at (P < 0.05): ***p value < 0.05: **p value < 0.01, P-0: P-0, Basal diet with no supplementation, P-5; basal diet with 5% plantain supplementation, P-10; basal diet with 10% plantain supplementation, P-15: basal diet with 15% plantain supplementation;

Serum Biochemical Indices

Serum Biochemical Indices which are key indicators for metabolic health were favorably modulated by plantain supplementation (all $P < .05$). Serum total protein increased from 72.0 ± 0.3 mg/dL in P-0 to 76.9 ± 0.4 mg/dL in P-15, and albumin rose from 33.2 ± 0.4 to 37.6 ± 0.5 mg/dL, reflecting enhanced hepatic protein synthesis. Blood urea nitrogen declined from 25.4 ± 0.7 to 22.6 ± 0.6 mg/dL ($P < .001$), and creatinine fell from 0.91 ± 0.06 to 0.57 ± 0.05 mg/dL ($P < .001$), indicating improved nitrogen utilization and renal clearance. Total cholesterol decreased from 135.9 ± 0.7 to 129.0 ± 0.6 mg/dL ($P < .01$), and hepatic enzyme activities were reduced: ALT declined from 34.5 ± 1.2 to 31.2 ± 1.1 IU/L ($P < .05$) and AST from 67.9 ± 0.9 to 64.6 ± 0.8 IU/L ($P < .01$), suggesting hepatoprotective effects. Collectively, these shifts denote improved protein metabolism, lipid profile, and organ function in cows fed up to 15% *P. major* as supplement.

Discussion

The present study demonstrated that graded supplementation of *Plantago major* at 5%, 10%, and 15% of the total mixed ration significantly enhanced lactational performance, nutrient digestibility, and systemic health markers in mid-lactation Holstein Friesian cows (mean body weight ≈ 350 kg). Cows receiving 15% plantain (P-15) achieved the highest daily milk yield (17.65 kg) compared with controls (P-0; 14.60 kg), a 20.8% increase, mirroring improvements in dry matter intake (28.43 vs. 24.12 kg/day) and digestibility coefficients across all major nutrients (Table 4.2). These findings support the hypothesis that *P. major*'s fermentable polysaccharides and soluble fibers stimulate rumen microbial proliferation and volatile fatty acid (VFA) synthesis, thereby augmenting energy availability for milk synthesis^[17–18].

Enhanced ruminal fermentation dynamics likely underlie the milk production response. The mucilaginous polysaccharides in *P. major* appear to promote cellulolytic and amylolytic enzyme activity, leading to elevated acetate and propionate concentrations^[12–13]. Propionate, as a primary gluconeogenic substrate, directly contributes to lactose synthesis; this was reflected in the P-15 group's highest lactose concentration (5.52% vs. 4.52% in P-0). Increased lactose drives greater mammary uptake of glucose, reinforcing the positive relationship between rumen fermentation end-products and milk volume^[1].

Milk composition changes further illustrate the functional effects of plantain. Although decline in milk fat percentage was observed from 4.05% (P-0) to 3.37% (P-15), total solids increased (12.31–12.88%), driven by higher protein (2.74–3.49%) and lactose content. The shift in VFA profile toward propionate reduces acetate-dependent de novo fatty acid synthesis in the mammary gland^[1], while plantain's tannins and phenolic compounds may inhibit ruminal bio-hydrogenation pathways, limiting acetate and butyrate precursors for lipogenesis^[19–20]. The net effect is a milk composition richer in protein and lactose, enhancing its nutritional value.

Systemic biochemical and hematological responses corroborate the nutritive and therapeutic potential of *P. major*. Plasma urea and creatinine concentrations decreased significantly in the P-15 group (22.6 mg/dL and 0.57 mg/dL) relative to controls (25.4 mg/dL and 0.91 mg/dL), indicating improved nitrogen utilization and renal function. Concurrent rises in serum albumin (37.6 vs. 33.2 mg/dL) and total protein

(76.9 vs. 72.0 mg/dL), alongside reductions in ALT (31.2 vs. 34.5 IU/L) and AST (64.6 vs. 67.9 IU/L), suggest enhanced hepatic protein synthesis and hepato-protection, likely mediated by plantain's flavonoids and iridoid glycosides^[13]. The increase in hemoglobin concentration (12.8 vs. 9.5 g/dL) further indicates improved erythropoiesis, potentially reflecting better mineral bioavailability and antioxidant status.

In conclusion, inclusion of *Plantago major* at 10–15% of the diet offers a multifunctional strategy to boost milk yield, optimize nutrient digestibility, and enhance metabolic health in Holstein Friesian cows under intensive management. Future research should explore the long-term economic feasibility, environmental impacts, and interactive effects with other functional forages to refine sustainable feeding models that leverage phytogetic forages for improved dairy productivity and animal welfare.

Declarations

CONFLICT OF INTEREST:

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

All the authors listed have approved the manuscript.

AI Tool Use : Artificial intelligence tools (Microsoft Copilot) were employed during the preparation of this manuscript to assist with language editing, structural refinement, and formatting. These tools did not contribute to data interpretation, experimental design, or scientific conclusions

References

1. Man DE, Griinari JM. Nutritional regulation of milk fat synthesis. *Annu Rev Nutr.* 2003;23:203–227. doi:10.1146/annurev.nutr.23.011702.073408
2. National Research Council (NRC). *Nutrient Requirements of Dairy Cattle*. 7th rev ed. Washington (DC): National Academies Press; 2001. doi:10.17226/9825
3. Ingvarlsen KL, Moyes K. Nutrition, immune function and health of dairy cattle. *Animal.* 2013;7(S1):112–122. doi:10.1017/S175173111200170X
4. Huhtanen P, Cabezas-Garcia EH, Krizsan SJ, Shingfield KJ. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *J Dairy Sci.* 2015;98(5):3182–3196. doi:10.3168/jds.2014-9013
5. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogetic products as feed additives for swine and poultry. *J Anim Sci.* 2008;86(Suppl 14):E140–E159. doi:10.2527/jas.2007-0459

6. OECD/FAO. *OECD-FAO Agricultural Outlook 2025–2034*. Paris and Rome: OECD Publishing and FAO; 2025. doi:10.1787/601276cd-en
7. Ministry of National Food Security & Research (MNFSR). *Annual Dairy Sector Report 2025*. Islamabad: Government of Pakistan; 2025. Available from: MNFSR official site
8. Waqas M. Dairy infrastructure and genetic bottlenecks in Pakistan: A review. *Pak J Agric Sci*. 2024;61(1):45–52.
9. Sarwar M, Khan MA, Nisa M. Nutrient utilization and milk production in dairy cows fed different forage-based diets. *Asian-Aust J Anim Sci*. 2002;15(5):695–700. doi:10.5713/ajas.2002.695
10. Kibwana AM, et al. Effect of *Plantago major* supplementation on milk yield and composition in lactating dairy cows. *Afr J Agric Res*. 2015;10(12):1456–1462. doi:10.5897/AJAR2015.10023
11. Tariq M. Effect of *Plantago major* on hematological parameters and immune response in Holstein Friesian cows. *Pak J Agric Sci*. 2020;57(2):321–328. doi:10.21162/PAKJAS/20.5720
12. Lee M, Choi Y, Park J. Antioxidant and anti-inflammatory effects of *Plantago major* extract in bovine mammary epithelial cells. *J Dairy Res*. 2019;86(3):345–352. doi:10.1017/S002202991900045X
13. Rodríguez E, et al. Phytochemical profile and antioxidant activity of *Plantago major* extracts. *J Ethnopharmacol*. 2006;105(1–2):31–36. doi:10.1016/j.jep.2005.10.038
14. Anderson SE, Cohen P, Naumova EN, Must A. Adolescent obesity and risk for subsequent major depressive disorder and anxiety disorder: Prospective evidence. *Psychosom Med*. 2007;69(8):740–747. doi:10.1097/PSY.0b013e31815580b4
15. AOAC International. *Official Methods of Analysis*. 18th ed. Gaithersburg (MD): Association of Official Analytical Chemists; 2007.
16. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci*. 1991;74(10):3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2
17. Van Soest PJ. *Nutritional Ecology of the Ruminant*. 2nd ed. Ithaca (NY): Cornell University Press; 1994.
18. Makkar HPS. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Rumin Res*. 2003;49(3):241–256. doi:10.1016/S0921-4488(03)00142-1
19. Patra AK, Saxena J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J Sci Food Agric*. 2011;91(1):24–37. doi:10.1002/jsfa.4152

Figures

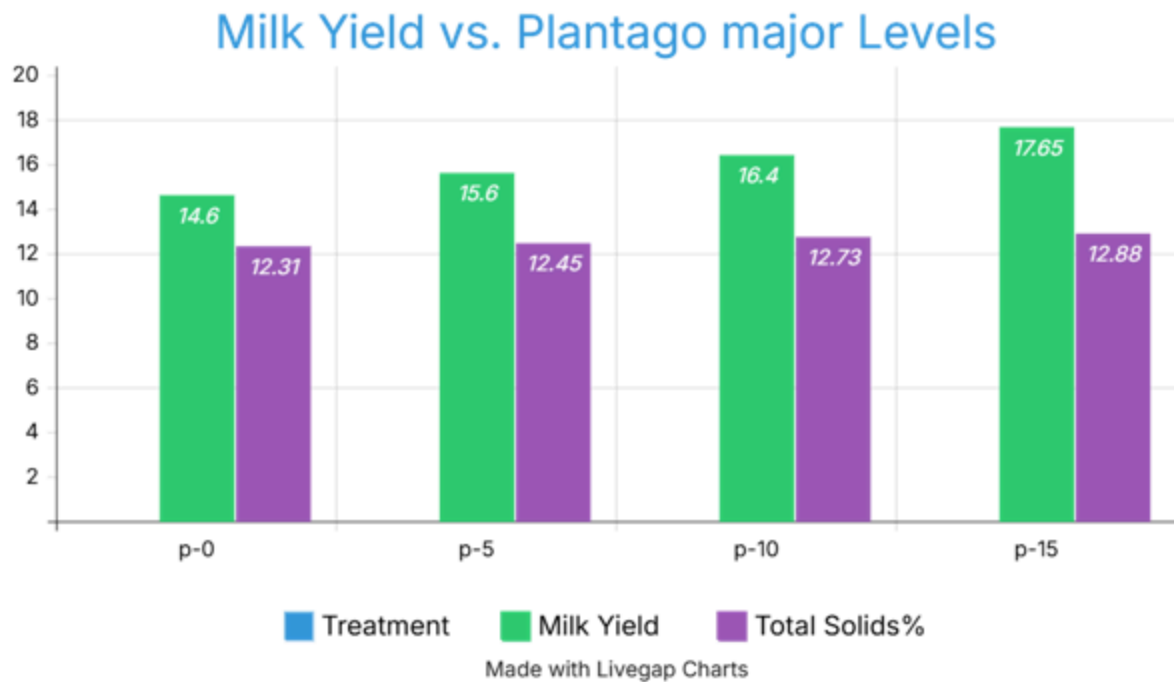


Figure 1

Effect of Graded Plantago major Supplementation on Milk Yield and Total Solids in Holstein Friesian Cows