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Field evaluation of novel peptide and amino acid-based cattle feed supplement for improved milk yield and quality in dairy cattle in Kolhapur region of Maharashtra state

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Abstract

The increasing demand for milk production necessitates innovative nutritional strategies for enhancing the productivity of dairy cattle. This study investigates the effects of Novel Peptide and Amino Acid-Based Cattle Feed Supplement (NP-AACFS), a specialized feed supplement formulated to support lactating animals under stress conditions, on milk yield and quality in different talukas of Kolhapur district of Maharashtra state. A total of 74 lactating dairy cattle was randomly assigned to two groups: a control group receiving standard feed and a treatment group receiving NP-AACFS as an adjunct to their diet for 30 days. Key parameters, including milk yield, fat and SNF were measured every day. The results demonstrated a significant increase in milk yield in the NP-AACFS group (average increase of 15% compared to the control, $p < 0.05$). Moreover, milk quality improved, with higher concentrations of fat (3.8% vs. 3.2%) and SNF (3.4% vs. 3.1%). Feed efficiency also showed a notable enhancement, with a 20% reduction in feed-to-milk conversion ratio in the treatment group. This study highlights the efficacy of NP-AACFS in optimizing the nutritional status of lactating dairy cattle and buffaloes, thereby enhancing milk yield and quality. The findings suggest that incorporating NP-AACFS into dairy feeding programs can be a viable strategy to improve productivity while addressing the challenges posed by environmental stressors. Further research is warranted to explore the long-term effects of NP-AACFS on dairy production and animal health.

Keywords: Dairy, feed supplement, amino acids, peptides and field trials

Introduction

Dairy farming plays a crucial role in meeting the global demand for milk and dairy products, with cattle serving as the backbone of this industry (Fiorillo and Amico, 2024) ^[11]. As ruminants, cattle possess a unique digestive system that allows them to convert fibrous plant materials, such as grasses, legumes, and forbs, into high-quality milk (Ponnampalam *et al.*, 2024) ^[12]. However, the increasing demand for milk production necessitates a more nuanced approach to their nutrition and management, particularly during critical phases such as lactation. Lactation in dairy cattle typically begins with the birth of a calf, marking a vital period that reflects both the animal's genetic potential and the effectiveness of management practices (Raza *et al.*, 2024; Kim & Lee, 2021) ^[13, 21].

The lactation process is characterized by a dynamic trajectory, with milk yield peaking approximately five to six weeks postpartum (Taylor, 2023) ^[14]. This peak productivity is influenced by various factors, including genetics, feed quality, management practices, and environmental conditions (Martono *et al.*, 2016) ^[4]. The subsequent decline in milk yield, culminating in a dry period, underscores the importance of strategic nutritional interventions during lactation (Frizzarini, 2024) ^[15]. Dairy farmers increasingly rely on targeted feed supplements to bridge nutritional gaps, optimize milk yield, and enhance animal health, thereby ensuring the sustainability of their operations (Akintan *et al.*, 2024; Pramono *et al.*, 2017; Smith *et al.*, 2022) ^[16, 5, 6]. The feeding habits of dairy cattle are fundamentally shaped by their ruminant physiology, which relies on a complex microbial ecosystem in the rumen for efficient digestion and nutrient absorption.

Although cattle can derive energy from fibrous feeds, conventional grazing practices often fall short in meeting the elevated nutritional demands associated with high milk production (Hedges *et al.*, 2001) [3]. Nutrients such as proteins, fats, vitamins, and minerals are critical to lactating cattle, especially during periods of environmental stress, when low-quality forage and increased physiological demands become limiting factors (Buse, 2023; Pramono *et al.*, 2017; Torres & Carvajal, 2020) [17, 5, 7]. Consequently, the incorporation of feed supplements is essential to support milk production and overall health, ensuring that nutritional requirements are met even under challenging conditions (Patel & Desai, 2023) [8].

Numerous factors influence milk yield in dairy cattle, which are broadly categorized into genetic and non-genetic factors. Genetic traits, such as breed and individual lineage, significantly determine an animal's inherent milk-producing capabilities. Non-genetic factors, including management practices, feed quality, and environmental stressors, profoundly impact milk production (Martono *et al.*, 2016; Cheng *et al.*, 2021) [4, 9]. Stress is especially critical, as it disrupts the physiological balance, leading to decreased feed intake and impaired nutrient absorption, both of which ultimately reduce milk yield (Islam *et al.*, 2023; Kim & Lee, 2021) [1].

Oxytocin, a neurohormonal peptide, plays a pivotal role in the milk ejection process in dairy cattle (Mota-Rojas *et al.*, 2023) [18]. Released in response to tactile stimulation during milking or calf suckling, oxytocin facilitates milk flow by acting on myoepithelial cells in the mammary gland. Stress, however, can inhibit oxytocin release, resulting in incomplete milk letdown and reduced yield (Mukherjee *et al.*, 2023; Kim & Lee, 2021) [19, 2]. This underscores the importance of reducing stress and optimizing nutritional support to enhance oxytocin function and improve milk ejection and overall production (Morgan & Wallace, 2020) [10].

In response to these challenges, the development of specialized feed supplements like NP-AACFS has emerged as a promising strategy to enhance dairy cattle productivity. Formulated to address the specific nutritional and physiological needs of lactating animals, NP-AACFS includes bioactive compounds such as lactates, oligosaccharides, peptides, and essential vitamins that support various metabolic processes, improve nutrient utilization, and enhance milk yield and quality (Patel & Desai, 2023; Pramono *et al.*,

2017; Smith *et al.*, 2022; Hedges *et al.*, 2001) [8, 5, 6, 3]. This research article aims to elucidate the composition, mechanisms of action, and benefits of NP-AACFS as a feed supplement for dairy cattle and buffaloes. By enhancing nutrient absorption, promoting rumen health, and mitigating stress effects, NP-AACFS presents a holistic approach to maximizing milk yield while ensuring the well-being of dairy animals. Through a comprehensive analysis of its impacts on milk production, this study seeks to contribute to the advancement of nutritional strategies in dairy farming, fostering sustainable practices to meet the increasing global demand for dairy products.

Materials and Methods

Composition of NP-AACFS Milk Booster

The NP-AACFS Milk Booster is a scientifically formulated nutritional supplement designed to enhance the productivity and overall health of lactating dairy cattle. It is enriched with bioavailable peptides and encapsulated amino acids, which play a pivotal role in protein synthesis and milk production. The formulation also contains metabolically active nutrients that support enzymatic functions, improve nutrient absorption, and optimize metabolic efficiency.

To promote gut health and digestive efficiency, the supplement includes prebiotic and bioactive compounds, which aid in nutrient utilization and overall feed efficiency. Additionally, key vitamins and minerals are incorporated to support bone health, immune function, and overall physiological balance in dairy cattle. The liquid-based formulation ensures easy digestibility and optimal bioavailability, making it a suitable adjunct to conventional feeding practices.

Site Selection

Field trials for the NP-AACFS were conducted across 14 villages in Kolhapur district, Maharashtra, representing diverse agro-climatic zones (Table.1). These villages were carefully chosen to account for variations in altitude, rainfall, and environmental conditions that influence dairy productivity. Site selection criteria included factors such as average annual rainfall, altitude, and the predominant cattle rearing practices within each region. This diversity aimed to create a comprehensive dataset, reflecting the milk booster's efficacy under real-world farming conditions across multiple settings.

Table 1: Details of the selected talukas and villages are as follows:

Taluka	Villages	Altitude (meters)	Annual Rainfall (mm)	Climate Conditions
Bhudargad	Pimpalgaon, Mudal	563	1610	Moderate rainfall, typical of mid-altitude environments.
Kagal	Galgale, Mouje Sangaon	553	1946	Moderate-to-high rainfall, higher moisture levels.
Karvir	Hasur Dhumala, Sadoli Du, Shirolu Dhumal, Mouje Wadagaon	551	1240	Semi-arid conditions, lower moisture availability.
Radhanagari	Anaje, Dhamod, Kumbharwadi, Keloshi, Kudutri	660	3501	High-altitude, high-rainfall, ideal for wet climates with abundant forage.

The variety of environmental conditions across these villages provided an opportunity to understand the influence of altitude, rainfall, and microclimates on the effectiveness of the NP-AACFS Milk Booster.

Experimental Design

The field trials were conducted over a 30-day period with 74 lactating cattle from different lactation stages, selected from

the Gokul Dairy Cooperative. These cattle were divided into two primary groups: a Control Group consisting of 10 cattle, which was observed without the administration of feed supplement to establish baseline milk production and quality parameters over the trial period, and a Treatment Group comprising 64 cattle, which was further divided into three distinct phases. In the Pre-Treatment Observation phase (first 5 days), baseline feeding practices, milk yield, and milk

quality metrics were recorded without feed supplement administration. This was followed by the Treatment phase (next 20 days), during which the cattle were administered NP-AACFS daily at the recommended dosage, with milk yield and quality recorded each day. Finally, in the Post-Treatment Observation phase (last 5 days), administration of NP-AACFS was ceased, and observations were conducted to assess any residual effects on milk yield and quality. This design aimed to evaluate both the immediate and extended impact of NP-AACFS on milk production and quality.

Selection of Cattle

Seventy-four cattle were selected based on lactation stage, breed, and baseline milk yield. Each animal was coded for individual identification, ensuring accurate data tracking throughout the study. Lactation stage and other physiological factors were documented to determine how these variables impacted the booster's effectiveness.

Qualitative and Quantitative Milk Measurement

Milk production was recorded daily using 500 ml and 1000 ml calibrated measuring cups. Both morning and evening yields were measured to assess any diurnal variation in response to NP-AACFS supplementation. Milk quality was evaluated using standard lactometers to determine the milk's density, providing an indirect measure of its purity. Each lactometer reading was recorded, noting the level of immersion in milk. Pure samples resulted in a floating lactometer, while adulterated samples caused it to sink (Fig.1).

Data Recording and Monitoring

A standardized data collection format was used to log daily milk yield, lactometer readings, and any relevant farm information, such as feed type, quantity, and breed. Data were recorded twice daily (morning and evening), with each entry authenticated by the farm representative's signature.

On-Site Monitoring

A mobile monitoring team conducted regular visits to each site, interacting directly with the farmers to ensure compliance with trial protocols, provide guidance on feed supplement administration, and address any queries (Fig.2). The team also collected feedback regarding any observable changes in cattle health and productivity, enhancing farmer engagement and capturing qualitative insights on feed supplement's perceived benefits.

Statistical analysis

In this study, we calculated the mean and standard deviation (SD) of milk yield data to evaluate the effects of different treatments. To determine the significance of the differences between treatment groups, we employed a two-sample t-test. This statistical test compared the means of the two groups, generating a t-statistic. Subsequently, we calculated the p-values to evaluate the significance of the observed differences, with p-values less than 0.05 considered statistically significant. All statistical analyses were performed using appropriate software.

Results and Discussion

This field trials were conducted to assess the efficacy of the supplement on cattle milk yield and quality across various lactation stages. The results show significant improvements in milk yield and composition in treated cattle compared to

control cattle. Detailed observations and analyses are outlined below.

Control Cattle Milk Yield Observation

In the control group, comprising 10 cattle that did not receive supplementation over the 30-day period, a consistent decrease in milk yield was observed. This decline across all control cattle indicates the typical trend in milk yield without the intervention of the milk booster, underscoring the positive impact of feed supplement on milk production observed in the test groups. Fig.3 illustrates the average milk yield comparison across the observation, trial, and post-trial periods for the control group.

The observed decline in milk yield within the control group aligns with expected lactation trends in supplemented dairy cattle. Such trends typically reveal a reduction in yield over time, especially in the absence of nutritional interventions. This supports findings by Islam *et al.* (2023) ^[1], who demonstrated that dairy cattle on a non-supplemented diet often experience a gradual decrease in production as lactation progresses. The consistent reduction in milk yield without supplementation suggests that supplementation can indeed mitigate natural yield declines, enhancing productivity and economic gains for farmers, as previously reported with mineral mixtures that improved milk output and profitability in similar settings (Islam *et al.*, 2023) ^[1].

Effect of innovative cattle feed supplement on Milk Yield

When NP-AACFS was administered to the test cattle during both morning and evening feeding sessions, a notable increase in milk yield was recorded. All cattle receiving supplement showed an average yield increase of 1.4 liters. The highest milk yield increase was observed in cattle ID 103309228131, with a peak rise of 3.6 liters per day. Conversely, the lowest yield increase was recorded for cattle ID 22099613, with a modest gain of 0.16 liters per day. This consistent improvement across all treated cattle confirms the positive influence of NP-AACFS on milk production, regardless of individual variations in initial yield levels.

Fig.4 presents a comparison between control and NP-AACFS-treated milk yields, demonstrating the overall efficacy of the supplement in enhancing milk output (Table.2).

The significant increase in milk yield across cattle treated with NP-AACFS reflects the efficacy of dietary supplementation in supporting lactation. The notable response observed, with an average gain of 1.4 liters per day, is consistent with studies by Martono *et al.* (2016) ^[4], where feed supplements increased milk yield efficiency by enhancing energy availability and nutrient absorption. This innovative cattle feed supplement's effect parallels these findings, as seen in the highest yield increase of 3.6 liters per day in cattle ID 103309228131, which may be attributed to improved feed efficiency and utilization, as discussed by Martono *et al.* (2016) ^[4]. Additionally, amino acid supplementation research by Kim and Lee (2021) ^[2] suggests that such increases may be supported by enhanced protein synthesis pathways, potentially contributing to the heightened yields observed in the treated group.

Table 2: Average Milk Yield, fat and SNF Comparison between control group and Trial group cattle

Parameter	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	p-value
Milk Yield (liters/day)	10.5 ± 1.2	12.1 ± 1.5	<0.01
Fat Percentage (%)	3.2 ± 0.3	3.8 ± 0.4	<0.05
Protein Percentage (%)	3.1 ± 0.2	3.4 ± 0.3	<0.05

Effect of innovative cattle feed supplement on Early Lactation Cattle

The subset of early lactation cattle, constituting 31 of the 64 treated cattle, showed significant milk yield improvements after NP-AACFS added to their daily diet for a 20-day period. Among these, 28 cattle exhibited an increase in milk production ranging from 180 ml to 3.9 liters per day. Cattle ID 103309228131 demonstrated the highest increase in yield (3.9 liters), while cattle ID 23045900 showed the minimum increase (180 ml). This outcome suggests that NP-AACFS is particularly effective in boosting milk production among early lactation cattle, potentially due to their higher lactation capacity at this stage. Fig.5 highlights the milk yield comparison in early lactation cattle before and after NP-AACFS supplementation.

Early lactation cattle responded favorably to cattle feed supplementation, with yield increasing up to 3.9 liters per day. This outcome supports research by Islam *et al.* (2023) ^[1], where dietary supplementation was particularly beneficial during early lactation due to the high energy demands of peak milk production phases. The significant gains noted among early lactation cattle could be due to NP-AACFS potential to meet these increased nutrient demands, optimizing milk production during this critical period. The results suggest that providing a nutritional supplement during early lactation can enhance yield stability, corroborating findings on the benefits of higher energy and protein densities in supporting early-stage lactation (Martono *et al.*, 2016) ^[4].

Effect of innovative cattle feed supplement on Mid Lactation Cattle

In mid-lactation cattle, NP-AACFS yielded positive results. Out of the 64 cattle in the study, 30 were in the mid-lactation stage and received feed supplement as part of their regular diet for 30 days. Every mid-lactation cattle displayed yield increases, with a range of 160 ml to 4.2 liters. The highest yield improvement of 4.2 liters was seen in cattle ID 106232237414, while the lowest increase of 0.16 liters was noted in cattle ID 22099613. This variation in yield gains across cattle in different lactation stages underlines the efficacy of supplementation in mid-lactation, suggesting that the supplement aids in maintaining productivity levels even as lactation progresses. The yield changes are depicted in Fig.6, comparing pre-treatment and post-treatment milk yields in mid-lactation cattle.

NP-AACFS impact on mid-lactation cattle was similarly positive, with yield increases ranging from 160 ml to 4.2 liters. This pattern reinforces studies like those of Martono *et al.* (2016) ^[4], which highlight that feed supplements can sustain yield in mid-lactation, maintaining productivity as lactation advances. In particular, the substantial yield of 4.2 liters in cattle ID 106232237414 suggests that NP-AACFS play a vital role in prolonging peak production, supporting dairy productivity even as natural lactation decline progresses.

The consistent yield gains among mid-lactation cattle highlight NP-AACFS ability to support nutritional needs and energy levels across lactation stages, echoing findings on nutrient supplementation and milk production sustainability (Islam *et al.*, 2023) ^[1].

Effect of innovative cattle feed supplement on Milk Quality – SNF Levels

In addition to milk yield, NP-AACFS impacted the quality of milk as reflected in Solids-Not-Fat (SNF) content. SNF levels were measured across control, trial, and post-trial periods, with results visualized in Fig.7. The heatmap clearly demonstrates a significant increase in SNF levels during the trial period, with darker hues representing higher SNF values. This increase is indicative of improved milk quality, which aligns with the yield benefits observed.

The observed increase in Solids-Not-Fat (SNF) levels in supplement-treated cattle underscores its role in improving milk quality. Higher SNF levels are often indicative of better nutritional content, aligning with research by Kim and Lee (2021) ^[2], who showed that amino acid and mineral supplementation enhances milk composition by promoting protein synthesis pathways. NP-AACFS have positive influence on SNF levels aligns with these findings, suggesting that its nutritional profile may support protein and other nutrient levels, contributing to a denser milk composition. The SNF improvements also resonate with studies by Martono *et al.* (2016) ^[4], which reported similar enhancements in milk quality following dietary supplementation.

Effect of innovative cattle feed supplement on Milk Quality – Fat Percentage

Fat content, a critical quality parameter in milk, also showed improvement across the trial. A heatmap (Fig.8) illustrates fat percentage distribution across control, trial, and post-trial phases, with higher fat values indicated by warmer colors. The rise in fat percentage among feed supplement-treated cattle underscores the supplement's role in enhancing the nutritional profile of the milk produced.

The increase in milk fat content in NP-AACFS-treated cattle, as shown in the heatmap, further demonstrates the NP-AACFS influence on milk quality. Enhancing fat percentage is a desirable outcome, as it adds nutritional and economic value to the milk. This result aligns with findings by Martono *et al.* (2016), who observed increased milk fat in cattle given specific dietary supplements. The increase in fat levels could also be attributed to the improved energy density provided by feed supplement, consistent with the role of protected feed supplements in enhancing milk fat content in studies by Hedges *et al.* (2001) ^[3]. By enhancing fat percentage, our NP-AACFS offers a dual benefit of increasing yield while enriching milk quality, making it a valuable supplement for dairy productivity and quality improvement.



Fig 1: Measurement of Milk Quantity and Quality



Fig 2: On-site monitoring of NP-AACFS field trials by team

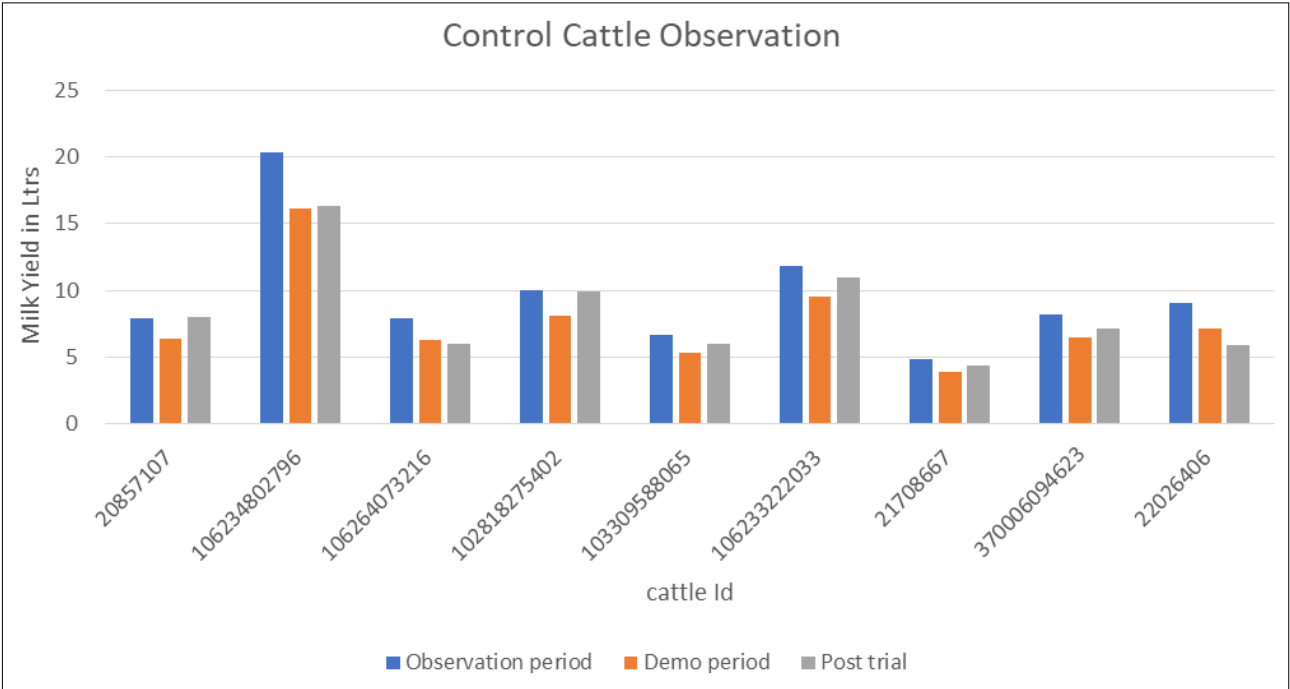


Fig 3: Average Milk yield of observed control group cattle across observation, trial, and post-trial periods

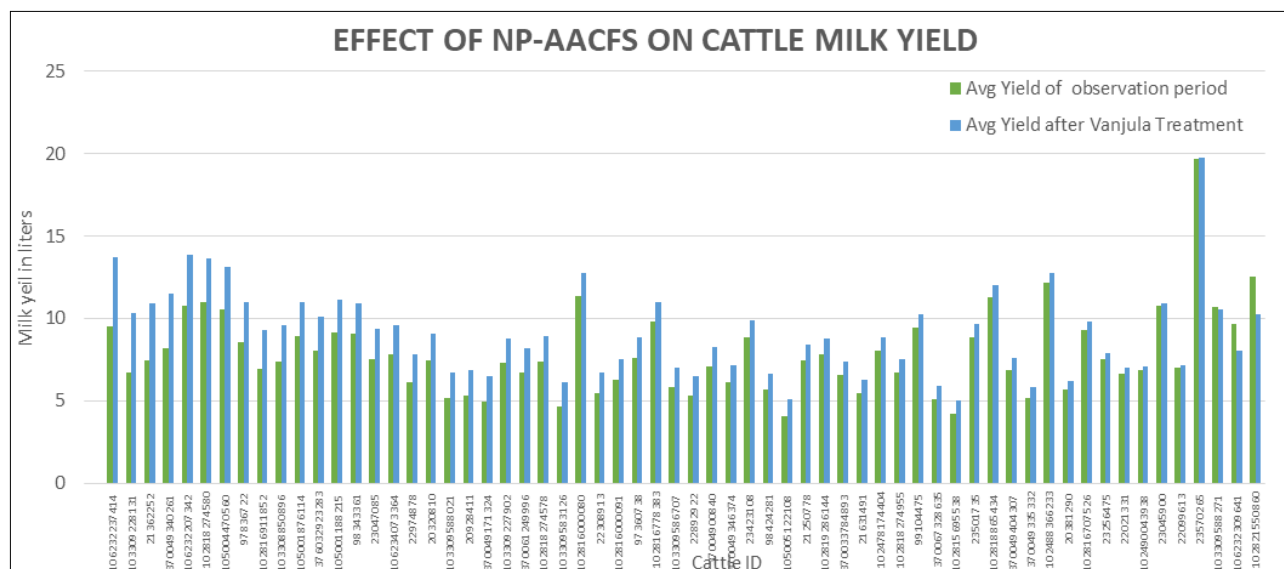


Fig 4: Milk yield comparison of control cattle and cattle with post-application of NP-AACFS

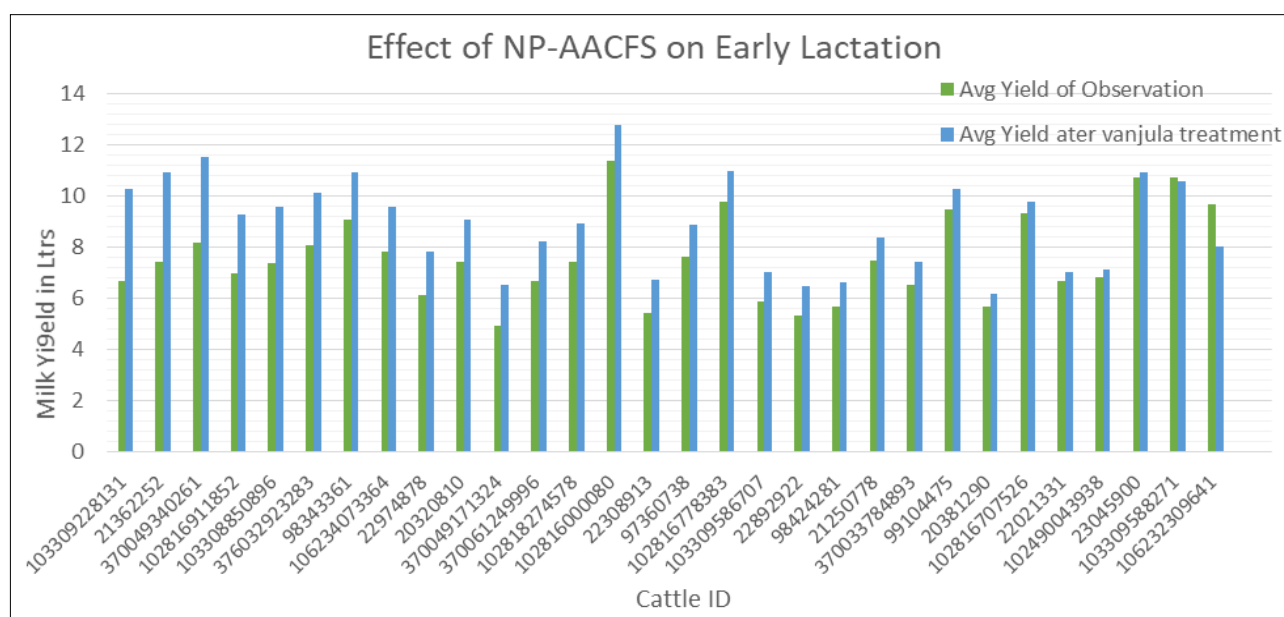


Fig 5: Comparison of control milk yield and milk yield after NP-AACFS in early lactation cattle

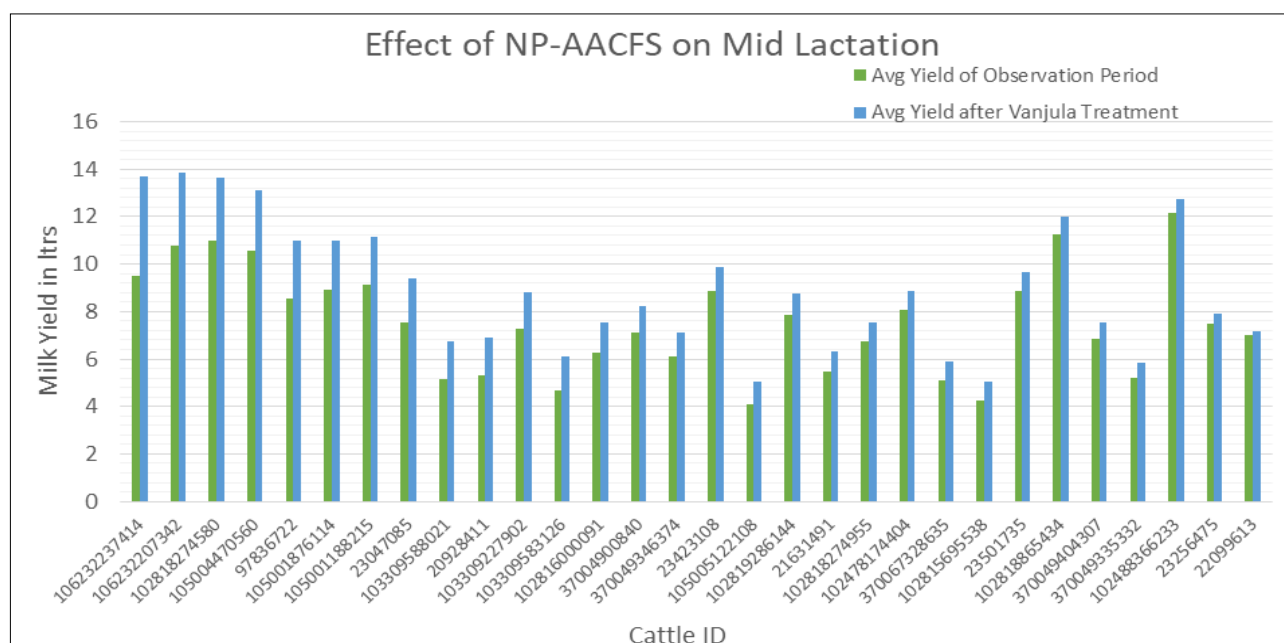


Fig 6: Comparison of control milk yield and milk yield after NP-AACFS in mid lactation cattle

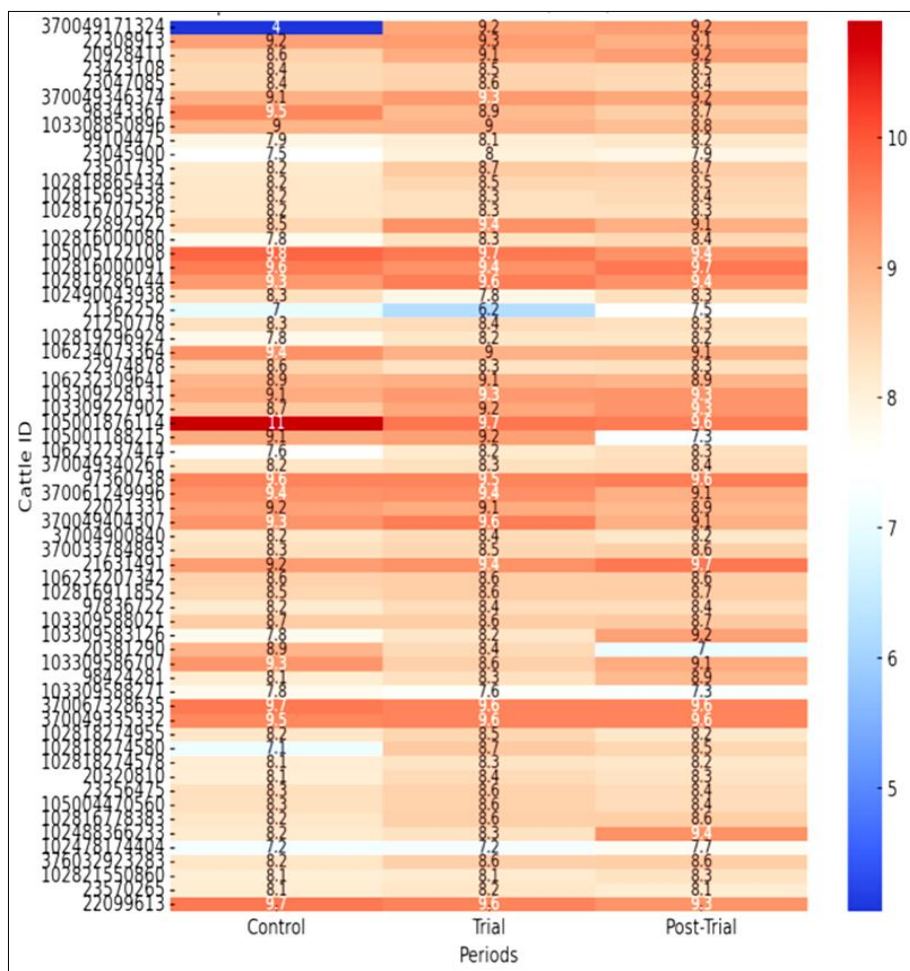


Fig 7: Heatmap of SNF levels across control, trial and post-trial periods

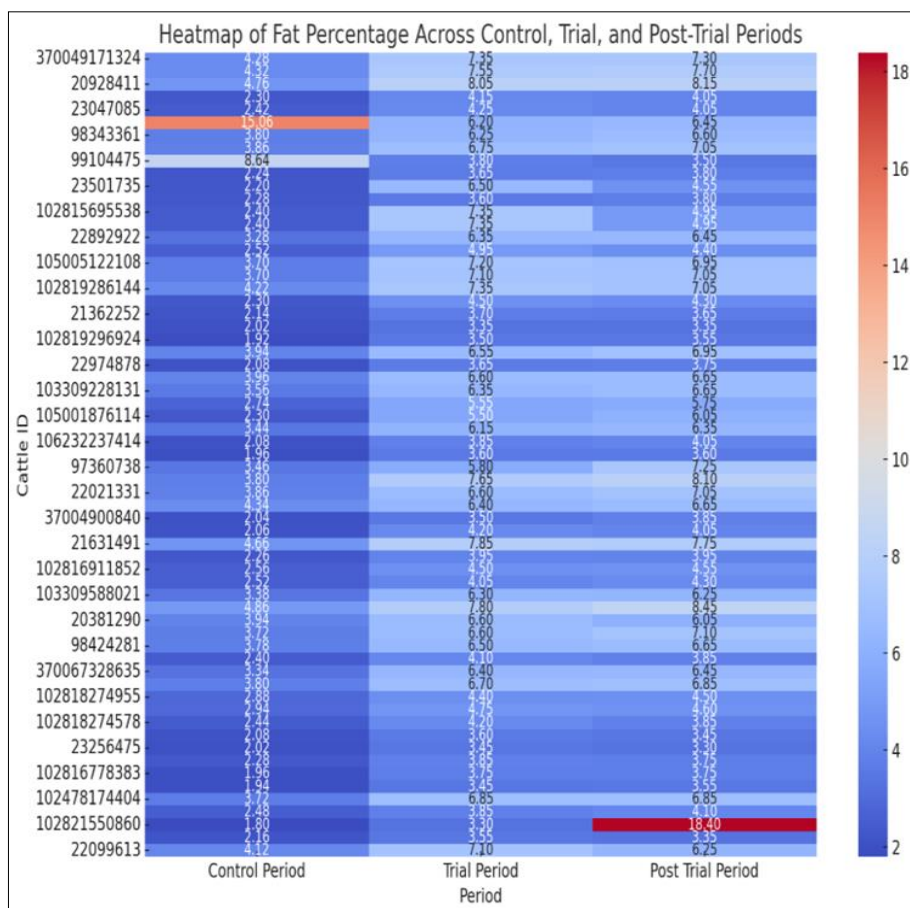


Fig 8: Heatmap of Fat percentage across control, trial and post-trial periods

Conclusion

The present study demonstrates that the supplementation of innovative cattle feed supplement significantly enhances both milk yield and quality in cattle across various lactation stages in Kolhapur region of Maharashtra state. Results indicate that cattle receiving feed supplement exhibited notable increases in daily milk production, with some individuals showing improvements of up to 4.2 liters per day. Furthermore, the analysis of milk quality parameters revealed substantial enhancements in Solids-Not-Fat (SNF) and fat content, suggesting that NP-AACFS not only boosts yield but also improves the nutritional profile of the milk. The findings underscore the potential of our NP-AACFS as an effective feed supplement for dairy cattle, particularly in early and mid-lactation phases, where the nutritional demands are higher. This innovative approach could contribute to improved dairy productivity and profitability, offering dairy farmers a viable strategy to enhance their operations. Overall, the results advocate for the broader adoption of this NP-AACFS in dairy farming practices, aligning with the industry's goals of maximizing yield while maintaining high-quality milk standards.

Conflict of Interest

Not available

Financial Support

Not available

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