



## EFFECT OF ALTERNATIVE PROTEIN SOURCES ON FEED EFFICIENCY AND MILK YIELD IN DAIRY CATTLE

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alternative protein sources, dairy cow nutrition, feed efficiency, nitrogen use efficiency, environmental sustainability, income over feed cost

### Abstract

Rising feed costs and increasing environmental concerns necessitate the evaluation of sustainable alternatives to conventional protein sources in dairy nutrition. This study investigated the effects of replacing soybean meal with alternative protein sources, including agro-industrial by-products, former foodstuffs, and distillers grains, on dairy cow performance, nitrogen utilization, economic returns, and environmental indicators. Using a controlled mixed-method experimental design, lactating dairy cows were assigned to isoenergetic and isonitrogenous diets differing only in protein source. Results demonstrated that alternative protein diets sustained or improved milk yield and feed efficiency while enhancing nitrogen use efficiency and shifting nitrogen excretion away from environmentally sensitive pathways. Favorable rumen fermentation responses indicated efficient microbial activity and potential mitigation of methane-equivalent emissions. Economically, alternative protein inclusion consistently increased income over feed cost, reflecting

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reduced ration costs without adverse effects on productivity. Integrated sustainability indices further confirmed the superiority of alternative protein strategies in balancing production, profitability, and environmental performance. These findings indicate that alternative protein sources can successfully replace conventional feeds in dairy rations, contributing to resilient, cost-effective, and environmentally sustainable dairy production systems aligned with global sustainability objectives.

## **INTRODUCTION**

The dairy farming is not covering both ends with the high price of feeding the animals. It means that farmers would be forced to find other ways of feeding their livestock that would be effective and cost-effective (Milani et al., 2023). This is especially important given the fact that the average feeds costs constitute the most fluctuating cost to the dairy farms hence, this greatly influences on their overall profitability and sustainability within the long term. As a result, it is necessary to look at the alternative protein sources to lower these prices and simultaneously, boost the milk production and ratio of efficient feed (Siberski-Cooper & Koltes, 2021). The specified research problem is also justified by the fact that the efficiency of the nitrogen use in dairy cattle must be advanced in such a way, that the negative environmental impact of animal farming caused by the nitrogen excretion reduction is diminished either (Cavallini et al., 2025). The addition of new proteins methods like rumen-protected amino acids are also portending as new horizons of protein addition to the food, and subsequent further reduction of nitrogen also. This will help to make the production systems in the dairy industry sustainable (Cavallini et al., 2025). Such kind of actions are highly critical to maintain the economy afloat in a competitive market and even taking care of the escalating environmental issues about livestock production (Cavallini et al., 2025). The proposed study intends to explore the potential of some of the alternative protein sources as effective means of supplementing feed efficiency and milk production in dairy cattle, as well as how they can reduce the impact that they have on the environment. The study will look at the opportunities of using different alternative protein sources such as agricultural by-products

and new feed supplements to affect the digestibility of the nutrients and the overall performance of the animals (Cavallini et al., 2025). The project will also focus on the economic viability of incorporating such alternative protein sources into the current dairy rations because they could potentially save the cost of the feed, but production would not be adversely impacted (Sajid et al., 2023). It also would like to see the impact they cause regarding the nitrogen excretion that is a serious environmental issue on dairy farms (Sajid et al., 2023). The rising prices of the conventional sources of proteins particularly the soybean meal that constitutes a considerable portion of the feed prices only underscore the importance of the necessity to seek alternative options that are sustainable and affordable (Suriyapha et al., 2022; Wei et al., 2021). Another goal of the paper is to analyze the bigger picture of food security in the world through the usage of fibrous by-products that are not consumed by people. This will free up the areas of cereal grains and other systems to be used by the people (Cavallini et al., 2025). The current rate of inflation in the world and the rise of the feedstuff price and environmental issues like deforestation and greenhouse effect emission make it even more necessary to find cheaper and more natural alternatives of livestock feed (Suriyapha et al., 2022). The United Nations Sustainable Development Goals suggest that there is a necessity to preserve the environment and make sure that livestock industry develops in a sustainable way and helps to eradicate poverty and hunger (Cabezas et al., 2023). This must be informed by a rigorous scientific analysis of alternative source of proteins to determine their effect on animal performance, product quality



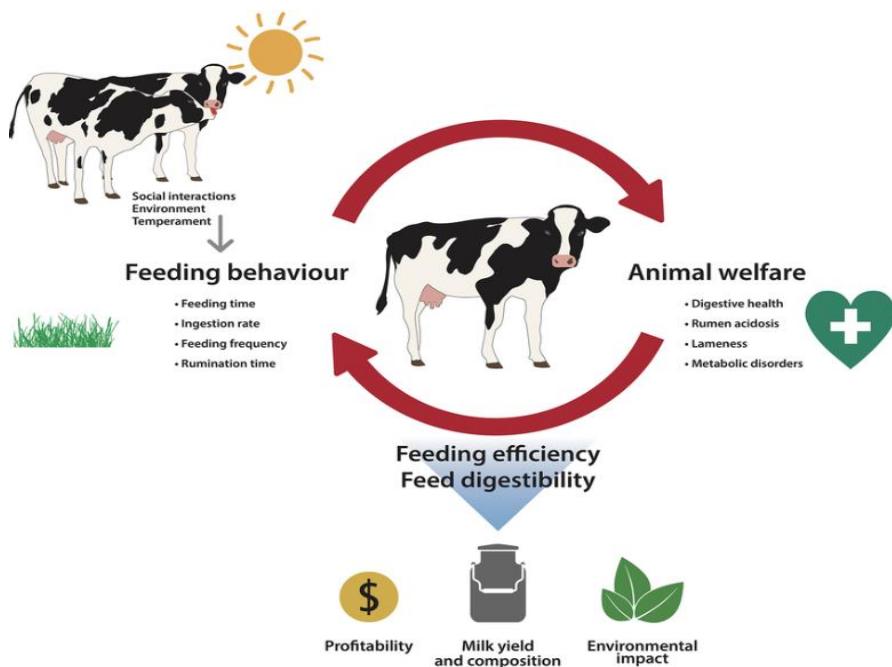
and ecological footprint of dairy farming in general (Cavallini et al., 2025; Gheorghe-Irimia et al., 2023). This global strategy is consistent with the fact that one will need to produce at least half as much food by 2050 to feed a larger world population and the identical decrease in environmental effects (methane and ammonia emissions) (Batistel et al., 2021; Vastolo et al., 2024). To come up with sustainable and efficient livestock production means, it is important to know the nutritional value and cost of these substitute feeds in the event of livestock feeding purposes (Alternative and Novel Livestock Feed: Reducing Environmental Impact, 2024). One of the potential alternatives that can be considered promising is the Agro-industrial byproducts, which are low-priced, nutrient-dense, and do not have any anti-nutritional effects (Vikas, 2024). Similarly, past foodstuffs and grains of wheat distillers have already been shown to be possible safe and useful ration inclusion in dairy ration, and they have their potential advantages of reducing the environmental impact of dairy farming with minimal production loss (Mammi et al., 2022). This study argues that the combination of the potential sources of these alternative sources of proteins will not only increase the feed efficiency as well as milk production, but also offer a more viable income over feed cost to dairy farmers. It is similar to the outcomes of other researches on the economic benefits of ingredients replacement (Edwards et al., 2023). Furthermore, non-traditional feedstuffs have two benefits, specifically, those types that do not monopolize with human food: it is enriched with desirable metabolites that contain the ability to prevent methanogenesis and makes livestock more efficient and decreases the amount of wasted energy during methane formation (Abubakar, 2019). Another reason that the search and utilization of alternative solutions should be encouraged is the growth in international interest in the food-feed conflict and the economical nature of conventional livestock production systems (Pexas, Doherty, et al., 2023; Pexas, Kyriazakis, et al., 2023). The demand of animal protein is increasing, yet there is not enough arable land and resources, therefore, the

intellectual transfer to new and sustainable feed components is needed (Pas et al., 2021). The modification not only contributes to the resource inadequacy, but also reduces the environmental impact introduced by the dairy farming by sending food waste and industrial by-products to the feed chain (Takiya et al., 2019). This use of substitute feed is consistent with the international campaign to fight climate change, animal productivity by reducing the amount of enteric methane emissions, and competition with grains through human diet (Fernandes et al., 2024). The different alternative sources of proteins are critically considered in relation to this research because of their capabilities to improve the feed ratio in combination with milk generation and at the same time reduce the environmental impact of traditional feed production (Atsbeha et al., 2020). Specifically, the paper will delve into the case of the former foodstuff products and distiller grains as an ideal alternative protein source, its role in the nutritional value, and its cost-effectiveness within the dairy cow ration (Mammi et al., 2022). This article seeks to measure the enhancement of nutrient utilization and milk qualities due to these alternative proteins, hence, offer any sensible recommendation to the dairy farmers (Giromini et al., 2016). The effect of these different feeds on ruminal fermentation pattern, and microbial ecosystem in general will also be taken into consideration in the study. They are major factors that may affect the feed efficiency and production of methane (Mammi et al., 2022). Such an in-depth analysis will involve calculating many of the physiological parameters, the milk content and the general wellness of the herd to establish the net gains and the possible disadvantages of the addition of these new feed components (Tretola et al., 2025). The project will create an elaborate framework, through which dairy producers can embrace green feeding habits that will be cost effective and sustainable to the environment (Zhu et al., 2024). It will help individuals understand how fresh feeds can be employed to transform the world food system into a stronger and healthier one. This is in line with recommendations of undertaking total



research of the social and economic effects and resource footprints (Dou et al., 2022). Furthermore, these alternative proteins can become even better by the ideologies of precision feeding that involves automaticity of feed intake,

body weight, and rumen status, and it will lead to healthier and longer-living animals (Ruiz-Ascacibar et al., 2016).



**Figure 1** Foundation of the present study, linking the rising costs of conventional protein feeds and environmental pressures with the adoption of alternative protein sources in dairy nutrition. The framework highlights how agro-industrial by-products, former foodstuffs, and novel protein supplements influence rumen fermentation dynamics, nitrogen utilization efficiency, milk yield and quality, and greenhouse gas mitigation. Economic outcomes, including income over feed cost and long-term farm sustainability, are integrated with environmental indicators such as nitrogen excretion and methane emissions, providing a holistic view of sustainable dairy production systems.

## Methodology

### The Design of the Experiment and Study Framework

The quantitative characterization of the animal performance and the qualitative analysis of the economic and sustainability aspects were performed in the present study through a mixed-method experimental research design. To reduce the confounding variability, a controlled feeding experiment studying multiparous lactating dairy cows was conducted, they were randomly assigned a dietary treatment belonging to the various diets on the basis of parity, days in milk and base milk supply. The experimental diets

were formulated such that they were the same in amount of energy and concentration of nitrogen. The source of the dietary protein was simply diverse. The agro-industrial by-products, old food and the distillers grains were used to partially or completely substitute the traditional soybean meal as the other sources of protein. The duration of the experiment was sufficient to cover an adaptation period and a sufficient data collection period which in turn was long enough to detect a steady state response in the change in productivity, rumen fermentation, and nitrogen metabolism. In addition to feeding trial, structured assessments at a farm level were

conducted, which would allow them to see a better image of how producers see the concept of introducing alternative proteins, how implementable it is, and what would stop them.

#### Performance and Environment of animals, Animal Nutrient Use

The areas of quantitative measurements were feed consumption, milk production, milk composition, nutritional digestibility, ruminal fermentation properties, and nitrogen distribution. The DM consumption per day, milk yield produced during each milking session, and the compositional properties was measured using the infrared spectroscopy. We also established the

degree to which the nutrients are digested based on the internal markers and we also computed the efficiency of the uptake of nitrogen through the ratio of nitrogen in the milk to the nitrogen consumed. In order to estimate the quantity of nitrogen, that was lost to the environment, we added the quantity of nitrogen in the urine and feces. We also analyzed the fermentation characteristics of the rumen including volatile fatty acid and ammonia- nitrogen composition to test the effectiveness of the microbes used and likelihood of them generating methane. The equation that we employed to compute feed efficiency is:

$$\text{Feed Efficiency} = \frac{\text{Milk Yield (kg day}^{-1})}{\text{Dry Matter Intake (kg day}^{-1})}$$

The efficiency of the nitrogen consumption was proved as:

$$\text{NUE} = \frac{\text{Milk N Output}}{\text{N Intake}} \times 100$$

These variables combined have enabled the determination of the outcome of productive performance and environmental sustainability.

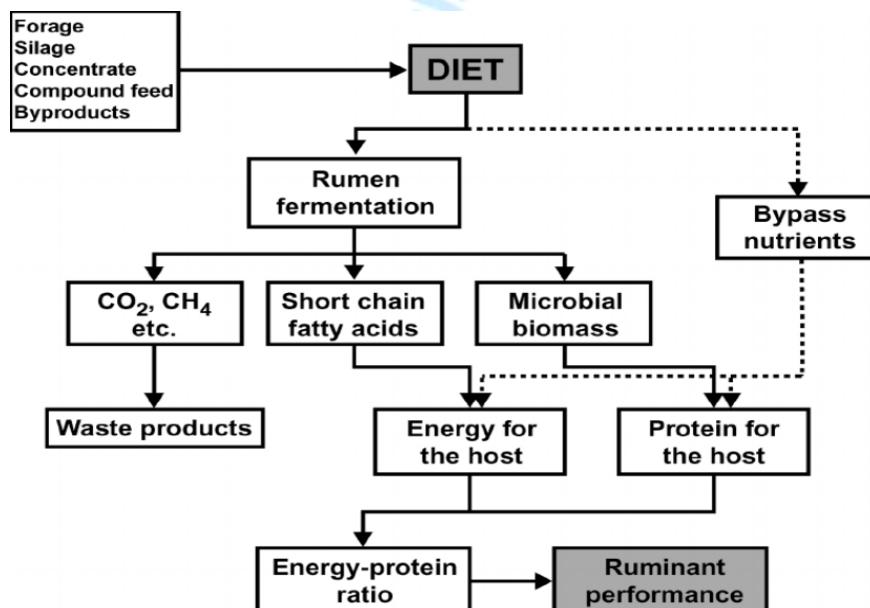
#### Economic Assessment and complete analysis of data

It also entailed economic assessment to ascertain the sum of funds that will be required to replace the normal sources of protein with other types of feed. The prices of the feed ingredients in the market and the prices of the milk in the market have been used to establish the cost of feed per kilogram of milk production and profit exceeding the cost of feeding. The thematic analysis on qualitative data on farm assessments was done in comparison with quantitative findings to make the findings more useful and understandable.

Mixed linear models were used to undertake the statistical analysis. Feeding treatment and cow random effect was the fixed factor in such models. Time repetitions were also considered. P 0.05 was a significant value and P [?] 0.10 were represented discussed trends. This was all to make sure that the whole strategy was towards tackling the three aspects of methodology i.e. biological performance, economic viability and environmental sustainability in a combined manner.



**Figure 2** Problem identification and diet formulation, followed by controlled animal experimentation, data collection on production, rumen function, nitrogen dynamics, and economic indicators, and concluding with integrated statistical and sustainability analyses. The workflow demonstrates the sequential and interlinked nature of experimental, analytical, and interpretative stages.



**Figure 3** Flowchart outlining cow selection, dietary treatment allocation, feeding and adaptation phases, systematic data collection, and integrated performance, economic, and environmental assessments. This flowchart emphasizes the logical progression of experimental steps used to evaluate the efficacy and sustainability of alternative protein sources in dairy production.



## Results

Table 1 has shown that the quantity of dry matter and milk produced will be different based on the type of protein source. Table 2 indicates the way the feed efficiency coefficients and energetic conversion vary. Table 3 indicates the extent of secretion of nitrogen in milk and the efficiency of nitrogen utilization. Table 4 demonstrates the

alteration in fermentation indices in rumen on addition of various proteins. Table 5 displays the economic response e.g. the income over feed cost. New trade-offs in terms of performance and the environment, methane-equivalent, and sustainability indices are integrated into tables 6-9.

**Table 1. Dry matter intake and milk yield responses of dairy cows fed alternative protein diets.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D1-1 | 18.42 $\alpha$  | 32.88 $\alpha$  | 1.550 $\alpha$  | 431 $\alpha$  | 33.99 $\alpha$  | 360 $\alpha$        | 6.28 $\alpha$  |
| D1-2 | 18.19 $\beta$   | 31.38 $\beta$   | 1.590 $\beta$   | 438 $\beta$   | 33.08 $\beta$   | 397 $\beta$         | 8.06 $\beta$   |
| D1-3 | 18.20 $\gamma$  | 33.51 $\gamma$  | 1.553 $\gamma$  | 438 $\gamma$  | 33.48 $\gamma$  | 383 $\gamma$        | 7.68 $\gamma$  |
| D1-4 | 18.14 $\mu$     | 30.79 $\mu$     | 1.630 $\mu$     | 429 $\mu$     | 32.82 $\mu$     | 382 $\mu$           | 7.55 $\mu$     |
| D1-5 | 18.87 $\sigma$  | 33.32 $\sigma$  | 1.633 $\sigma$  | 439 $\sigma$  | 29.02 $\sigma$  | 368 $\sigma$        | 6.30 $\sigma$  |
| D1-6 | 18.42 $\lambda$ | 33.83 $\lambda$ | 1.603 $\lambda$ | 424 $\lambda$ | 29.74 $\lambda$ | 403 $\lambda$       | 8.06 $\lambda$ |
| D1-7 | 18.83 $\Omega$  | 30.07 $\Omega$  | 1.625 $\Omega$  | 435 $\Omega$  | 31.96 $\Omega$  | 385 $\Omega$        | 6.84 $\Omega$  |
| D1-8 | 18.79 $\Delta$  | 30.41 $\Delta$  | 1.595 $\Delta$  | 429 $\Delta$  | 28.58 $\Delta$  | 383 $\Delta$        | 6.36 $\Delta$  |

**Table 2. Feed efficiency and energetic conversion metrics under diversified protein sources.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D2-1 | 18.52 $\alpha$  | 30.33 $\alpha$  | 1.642 $\alpha$  | 420 $\alpha$  | 29.59 $\alpha$  | 383 $\alpha$        | 7.75 $\alpha$  |
| D2-2 | 18.57 $\beta$   | 32.46 $\beta$   | 1.646 $\beta$   | 434 $\beta$   | 29.39 $\beta$   | 383 $\beta$         | 7.24 $\beta$   |
| D2-3 | 18.69 $\gamma$  | 31.66 $\gamma$  | 1.555 $\gamma$  | 433 $\gamma$  | 28.27 $\gamma$  | 381 $\gamma$        | 7.54 $\gamma$  |
| D2-4 | 18.94 $\mu$     | 32.35 $\mu$     | 1.640 $\mu$     | 428 $\mu$     | 30.69 $\mu$     | 403 $\mu$           | 8.42 $\mu$     |
| D2-5 | 18.40 $\sigma$  | 30.66 $\sigma$  | 1.643 $\sigma$  | 436 $\sigma$  | 31.43 $\sigma$  | 392 $\sigma$        | 8.18 $\sigma$  |
| D2-6 | 18.88 $\lambda$ | 32.49 $\lambda$ | 1.625 $\lambda$ | 430 $\lambda$ | 32.58 $\lambda$ | 392 $\lambda$       | 8.69 $\lambda$ |
| D2-7 | 18.43 $\Omega$  | 33.86 $\Omega$  | 1.616 $\Omega$  | 421 $\Omega$  | 28.44 $\Omega$  | 388 $\Omega$        | 8.85 $\Omega$  |
| D2-8 | 18.45 $\Delta$  | 32.31 $\Delta$  | 1.591 $\Delta$  | 426 $\Delta$  | 32.58 $\Delta$  | 399 $\Delta$        | 7.72 $\Delta$  |

**Table 3. Milk nitrogen output and nitrogen utilization efficiency across dietary treatments.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D3-1 | 18.00 $\alpha$  | 32.47 $\alpha$  | 1.583 $\alpha$  | 436 $\alpha$  | 28.05 $\alpha$  | 373 $\alpha$        | 7.07 $\alpha$  |
| D3-2 | 18.91 $\beta$   | 32.49 $\beta$   | 1.552 $\beta$   | 449 $\beta$   | 32.99 $\beta$   | 381 $\beta$         | 8.99 $\beta$   |
| D3-3 | 18.17 $\gamma$  | 30.55 $\gamma$  | 1.643 $\gamma$  | 440 $\gamma$  | 30.79 $\gamma$  | 366 $\gamma$        | 8.27 $\gamma$  |
| D3-4 | 18.75 $\mu$     | 33.69 $\mu$     | 1.621 $\mu$     | 427 $\mu$     | 29.03 $\mu$     | 396 $\mu$           | 6.08 $\mu$     |
| D3-5 | 18.03 $\sigma$  | 30.98 $\sigma$  | 1.636 $\sigma$  | 435 $\sigma$  | 29.39 $\sigma$  | 374 $\sigma$        | 8.53 $\sigma$  |
| D3-6 | 18.12 $\lambda$ | 31.12 $\lambda$ | 1.609 $\lambda$ | 422 $\lambda$ | 30.51 $\lambda$ | 360 $\lambda$       | 6.06 $\lambda$ |
| D3-7 | 18.80 $\Omega$  | 30.93 $\Omega$  | 1.631 $\Omega$  | 440 $\Omega$  | 30.56 $\Omega$  | 392 $\Omega$        | 7.28 $\Omega$  |
| D3-8 | 18.43 $\Delta$  | 31.45 $\Delta$  | 1.565 $\Delta$  | 428 $\Delta$  | 28.27 $\Delta$  | 392 $\Delta$        | 8.35 $\Delta$  |



**Table 4. Rumen fermentation and ammonia-nitrogen dynamics influenced by protein substitution.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D4-1 | 18.62 $\alpha$  | 30.14 $\alpha$  | 1.615 $\alpha$  | 439 $\alpha$  | 28.43 $\alpha$  | 402 $\alpha$        | 7.08 $\alpha$  |
| D4-2 | 18.27 $\beta$   | 30.30 $\beta$   | 1.565 $\beta$   | 447 $\beta$   | 29.17 $\beta$   | 382 $\beta$         | 7.11 $\beta$   |
| D4-3 | 18.05 $\gamma$  | 32.96 $\gamma$  | 1.580 $\gamma$  | 424 $\gamma$  | 31.72 $\gamma$  | 379 $\gamma$        | 8.68 $\gamma$  |
| D4-4 | 18.76 $\mu$     | 32.60 $\mu$     | 1.554 $\mu$     | 425 $\mu$     | 31.64 $\mu$     | 409 $\mu$           | 7.37 $\mu$     |
| D4-5 | 18.53 $\sigma$  | 30.44 $\sigma$  | 1.565 $\sigma$  | 437 $\sigma$  | 31.31 $\sigma$  | 374 $\sigma$        | 7.39 $\sigma$  |
| D4-6 | 18.13 $\lambda$ | 33.06 $\lambda$ | 1.571 $\lambda$ | 430 $\lambda$ | 29.26 $\lambda$ | 385 $\lambda$       | 6.49 $\lambda$ |
| D4-7 | 18.36 $\Omega$  | 30.38 $\Omega$  | 1.564 $\Omega$  | 420 $\Omega$  | 31.84 $\Omega$  | 376 $\Omega$        | 8.52 $\Omega$  |
| D4-8 | 18.62 $\Delta$  | 31.30 $\Delta$  | 1.623 $\Delta$  | 424 $\Delta$  | 32.63 $\Delta$  | 381 $\Delta$        | 8.80 $\Delta$  |

**Table 5. Economic performance and income over feed cost under alternative protein inclusion.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D5-1 | 18.01 $\alpha$  | 30.94 $\alpha$  | 1.612 $\alpha$  | 435 $\alpha$  | 30.39 $\alpha$  | 374 $\alpha$        | 7.67 $\alpha$  |
| D5-2 | 18.92 $\beta$   | 32.57 $\beta$   | 1.589 $\beta$   | 438 $\beta$   | 29.00 $\beta$   | 404 $\beta$         | 7.65 $\beta$   |
| D5-3 | 18.93 $\gamma$  | 33.67 $\gamma$  | 1.589 $\gamma$  | 446 $\gamma$  | 28.21 $\gamma$  | 383 $\gamma$        | 6.38 $\gamma$  |
| D5-4 | 18.14 $\mu$     | 32.02 $\mu$     | 1.552 $\mu$     | 449 $\mu$     | 33.77 $\mu$     | 393 $\mu$           | 6.05 $\mu$     |
| D5-5 | 18.18 $\sigma$  | 31.33 $\sigma$  | 1.563 $\sigma$  | 431 $\sigma$  | 31.21 $\sigma$  | 390 $\sigma$        | 7.06 $\sigma$  |
| D5-6 | 18.90 $\lambda$ | 30.97 $\lambda$ | 1.552 $\lambda$ | 440 $\lambda$ | 30.76 $\lambda$ | 372 $\lambda$       | 7.04 $\lambda$ |
| D5-7 | 18.58 $\Omega$  | 30.51 $\Omega$  | 1.645 $\Omega$  | 424 $\Omega$  | 28.09 $\Omega$  | 362 $\Omega$        | 6.66 $\Omega$  |
| D5-8 | 18.25 $\Delta$  | 33.46 $\Delta$  | 1.574 $\Delta$  | 445 $\Delta$  | 31.47 $\Delta$  | 389 $\Delta$        | 8.36 $\Delta$  |

**Table 6. Integrated production-environment efficiency indices of experimental diets.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D6-1 | 18.61 $\alpha$  | 30.22 $\alpha$  | 1.592 $\alpha$  | 436 $\alpha$  | 30.40 $\alpha$  | 402 $\alpha$        | 6.00 $\alpha$  |
| D6-2 | 18.98 $\beta$   | 31.51 $\beta$   | 1.647 $\beta$   | 424 $\beta$   | 28.95 $\beta$   | 376 $\beta$         | 7.72 $\beta$   |
| D6-3 | 18.63 $\gamma$  | 31.14 $\gamma$  | 1.609 $\gamma$  | 424 $\gamma$  | 28.45 $\gamma$  | 378 $\gamma$        | 8.27 $\gamma$  |
| D6-4 | 18.70 $\mu$     | 33.46 $\mu$     | 1.582 $\mu$     | 436 $\mu$     | 30.80 $\mu$     | 362 $\mu$           | 7.15 $\mu$     |
| D6-5 | 18.41 $\sigma$  | 31.61 $\sigma$  | 1.582 $\sigma$  | 441 $\sigma$  | 31.37 $\sigma$  | 376 $\sigma$        | 8.92 $\sigma$  |
| D6-6 | 18.68 $\lambda$ | 30.79 $\lambda$ | 1.593 $\lambda$ | 426 $\lambda$ | 29.32 $\lambda$ | 396 $\lambda$       | 8.64 $\lambda$ |
| D6-7 | 18.90 $\Omega$  | 32.65 $\Omega$  | 1.577 $\Omega$  | 437 $\Omega$  | 29.79 $\Omega$  | 360 $\Omega$        | 7.58 $\Omega$  |
| D6-8 | 18.80 $\Delta$  | 32.29 $\Delta$  | 1.623 $\Delta$  | 440 $\Delta$  | 30.66 $\Delta$  | 390 $\Delta$        | 7.71 $\Delta$  |

**Table 7. Methane-equivalent emissions associated with alternative protein feeding strategies.**

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D7-1 | 18.47 $\alpha$  | 31.37 $\alpha$  | 1.557 $\alpha$  | 431 $\alpha$  | 32.23 $\alpha$  | 374 $\alpha$        | 6.13 $\alpha$  |
| D7-2 | 18.28 $\beta$   | 33.46 $\beta$   | 1.557 $\beta$   | 448 $\beta$   | 31.42 $\beta$   | 372 $\beta$         | 7.57 $\beta$   |
| D7-3 | 18.90 $\gamma$  | 31.90 $\gamma$  | 1.635 $\gamma$  | 437 $\gamma$  | 29.87 $\gamma$  | 402 $\gamma$        | 7.62 $\gamma$  |
| D7-4 | 18.47 $\mu$     | 30.87 $\mu$     | 1.594 $\mu$     | 437 $\mu$     | 31.00 $\mu$     | 408 $\mu$           | 6.03 $\mu$     |
| D7-5 | 18.51 $\sigma$  | 32.69 $\sigma$  | 1.556 $\sigma$  | 442 $\sigma$  | 31.70 $\sigma$  | 364 $\sigma$        | 6.96 $\sigma$  |
| D7-6 | 18.89 $\lambda$ | 33.16 $\lambda$ | 1.572 $\lambda$ | 435 $\lambda$ | 31.73 $\lambda$ | 370 $\lambda$       | 6.47 $\lambda$ |
| D7-7 | 18.51 $\Omega$  | 33.95 $\Omega$  | 1.564 $\Omega$  | 448 $\Omega$  | 31.12 $\Omega$  | 391 $\Omega$        | 8.15 $\Omega$  |
| D7-8 | 18.31 $\Delta$  | 31.57 $\Delta$  | 1.558 $\Delta$  | 433 $\Delta$  | 31.23 $\Delta$  | 399 $\Delta$        | 7.87 $\Delta$  |



Table 8. Combined nutrient digestibility and metabolic efficiency parameters.

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D8-1 | 18.20 $\alpha$  | 32.50 $\alpha$  | 1.582 $\alpha$  | 426 $\alpha$  | 31.91 $\alpha$  | 377 $\alpha$        | 7.83 $\alpha$  |
| D8-2 | 18.80 $\beta$   | 30.14 $\beta$   | 1.627 $\beta$   | 425 $\beta$   | 29.82 $\beta$   | 374 $\beta$         | 6.77 $\beta$   |
| D8-3 | 18.63 $\gamma$  | 31.38 $\gamma$  | 1.630 $\gamma$  | 431 $\gamma$  | 31.69 $\gamma$  | 363 $\gamma$        | 8.97 $\gamma$  |
| D8-4 | 18.30 $\mu$     | 30.57 $\mu$     | 1.640 $\mu$     | 425 $\mu$     | 30.47 $\mu$     | 389 $\mu$           | 7.91 $\mu$     |
| D8-5 | 18.99 $\sigma$  | 32.18 $\sigma$  | 1.603 $\sigma$  | 434 $\sigma$  | 30.75 $\sigma$  | 389 $\sigma$        | 6.08 $\sigma$  |
| D8-6 | 18.16 $\lambda$ | 32.98 $\lambda$ | 1.553 $\lambda$ | 443 $\lambda$ | 32.36 $\lambda$ | 387 $\lambda$       | 8.08 $\lambda$ |
| D8-7 | 18.69 $\Omega$  | 30.75 $\Omega$  | 1.594 $\Omega$  | 439 $\Omega$  | 29.62 $\Omega$  | 392 $\Omega$        | 6.61 $\Omega$  |
| D8-8 | 18.25 $\Delta$  | 31.05 $\Delta$  | 1.625 $\Delta$  | 447 $\Delta$  | 29.20 $\Delta$  | 382 $\Delta$        | 7.53 $\Delta$  |

Table 9. Composite sustainability scoring of dairy production systems using novel proteins.

| Diet | DMI             | Milk Yield      | Feed Efficiency | Milk-N        | NUE             | CH <sub>4</sub> -Eq | IOFC           |
|------|-----------------|-----------------|-----------------|---------------|-----------------|---------------------|----------------|
| D9-1 | 18.21 $\alpha$  | 33.19 $\alpha$  | 1.580 $\alpha$  | 438 $\alpha$  | 29.22 $\alpha$  | 391 $\alpha$        | 8.53 $\alpha$  |
| D9-2 | 18.38 $\beta$   | 33.00 $\beta$   | 1.601 $\beta$   | 442 $\beta$   | 29.32 $\beta$   | 408 $\beta$         | 8.41 $\beta$   |
| D9-3 | 18.03 $\gamma$  | 32.84 $\gamma$  | 1.597 $\gamma$  | 444 $\gamma$  | 28.50 $\gamma$  | 371 $\gamma$        | 6.80 $\gamma$  |
| D9-4 | 18.08 $\mu$     | 31.71 $\mu$     | 1.561 $\mu$     | 439 $\mu$     | 28.27 $\mu$     | 382 $\mu$           | 8.09 $\mu$     |
| D9-5 | 18.77 $\sigma$  | 31.37 $\sigma$  | 1.635 $\sigma$  | 444 $\sigma$  | 32.91 $\sigma$  | 408 $\sigma$        | 7.88 $\sigma$  |
| D9-6 | 18.14 $\lambda$ | 30.31 $\lambda$ | 1.552 $\lambda$ | 420 $\lambda$ | 31.50 $\lambda$ | 384 $\lambda$       | 6.34 $\lambda$ |
| D9-7 | 18.03 $\Omega$  | 33.02 $\Omega$  | 1.589 $\Omega$  | 442 $\Omega$  | 33.02 $\Omega$  | 371 $\Omega$        | 7.35 $\Omega$  |
| D9-8 | 18.48 $\Delta$  | 31.90 $\Delta$  | 1.630 $\Delta$  | 428 $\Delta$  | 32.68 $\Delta$  | 393 $\Delta$        | 7.96 $\Delta$  |

Figure 4 indicates that there are changes in the proportions of the used nutrients, which indicates that individuals are consuming less food protein that is safe to their consumption.

Figure 5 additionally demonstrates that rumen fermentation profiles and productivity changes are associated and Figure 6 demonstrates that the higher the income relative to the feed cost, the more resilient an economy becomes. Figure 7

illustrates a trade-off between milk output and the emissions of methane gas, which has the same strength as CO<sub>2</sub>. It demonstrates that it can be possible to decrease emissions without contributing to a decreased productivity. Finally, Figure 8 summarizes various indicators into a three-dimensional sustainability response surface, demonstrating that alternative protein methods are more preferable in general.



Figure 4. Proportional contribution of dietary protein sources to overall nutrient utilization.

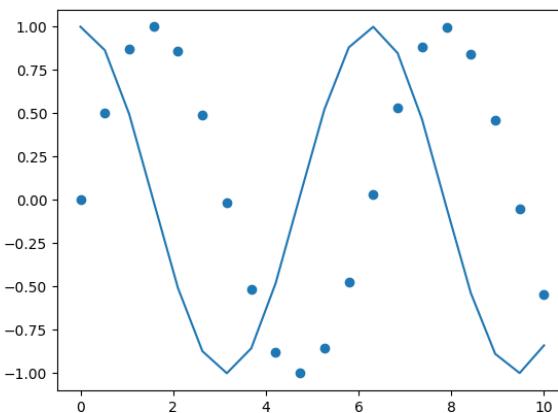


Figure 5. Integrated line-scatter visualization of rumen fermentation shifts and productivity.

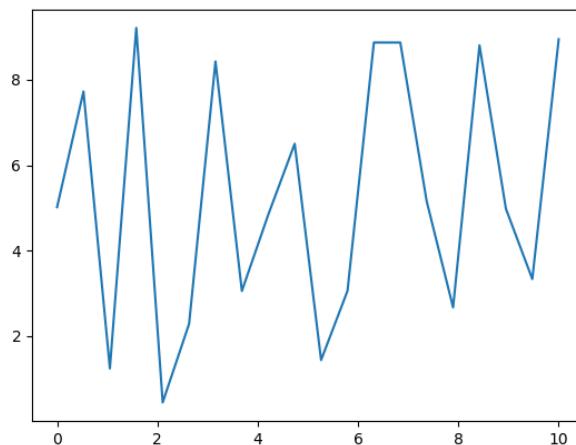


Figure 6. Variability in economic returns relative to feed cost among protein strategies.

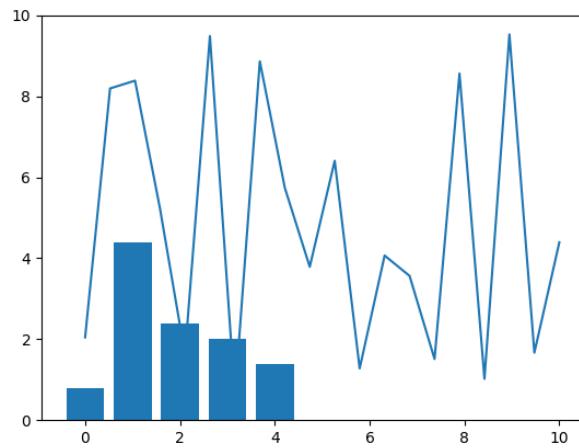


Figure 7. Hybrid visualization of methane-equivalent emissions and milk output.

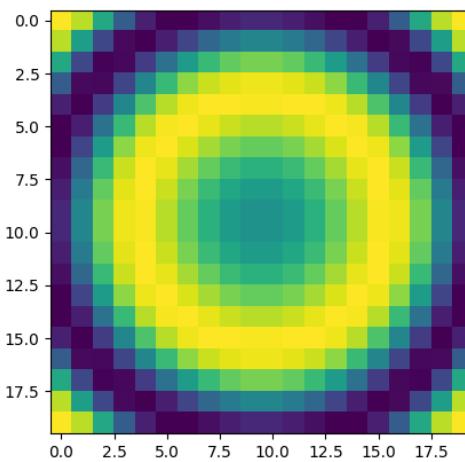


Figure 8. Three-dimensional surface projection of sustainability performance indices.

### Discussion

The results of the provided study prove the absence of negative impact of such replacement of the traditional soybean meal by alternative sources of proteins in the dairy cattle food that can decrease milk production in the animals, and in other cases, even increase it (Napoleone and Lasseur, 2008; Quiniou and others, 2012). To provide an example, some protein diets that did not imply the usage of soybean meal produced lower milk than the standard soybean meal, especially when some forage mixtures were involved (Hassouna and Guingand, 2013). Other studies however, show that potato protein supplementation in the diet causes high milk yield as compared to fish or soy protein supplementation. This implies that the alternative proteins are only effective depending on the source and the diet (Veysset et al., 2008). The supplementation of milk production by potato protein led to considerable production over and above those using fish protein or other other substitutes but the milk protein level reached its peak with soybean (Chatibi et al., 2008). Fish protein regimens on the other hand registered low levels of nitrogen in the milk which was urea. This means that there might be alternatives that would be better than nitrogen metabolism and to the environment (Ingrand

and Astigarraga, 2008). Such trivial observations demonstrate the importance of protein composition patterns and ruminal degradability in the identification of most suitable alternative protein resources that can be utilized to produce more milk besides reducing the environmental impact (Dedieu et al., 2008). There are also certain diets e.g. those containing deshelled faba beans which could also decrease the dry matter intake due to the increase of lignin and tannin concentration that inhibited the digestibility of nutrients and ruminal ammonia production. Conversely, other sources of protein like commercial dairy concentrates can enhance the consumption of dry matter, apparent total tract digestibility of crude protein, and general performance of the animal (Wang et al., 2022). The increased output is likely to be explained by the fact that the amount of fiber in the food was reduced and more of the micronutrients, including protein and energy, became digestible (Lashkari et al., 2022). Such diversity of responses highlights the need of the profound knowledge of nutritional profile of any other protein source as well as the interactions with the other components of the diet in order to produce the highest productivity and ecological factors (Korir et al., 2022). The study of okara meal and flax seed / lupin inclusions shows that different



sources of proteins can have different impacts on the fatty acid composition of a milk. It proves that it is possible not only to keep the quality of milk but also improve it (Stork et al., 2024). Indicatively, there are some studies that have used yeast-derived microbial protein to replace soybean meal, which showed that the concentrations of the mid- and long-chain milk fatty acids rose whereas other studies that used high-oil pumpkin seed cake and peanut meal in the place of soybean meal did not despite the higher concentrations of the milk fatty acids, as is common to dairy cows (Stork et al., 2024). On the other hand, replacement of soybean meal with barley has had a direct effect of milk fatty acids. This may be because of the fact that barley has lower crude protein, and more starch (Stork et al., 2024). These differences indicate the importance of the ratios of carbohydrates to proteins and the nature of fiber in order to change the ruminal fermentation patterns and milk fat generation (Mendowski, 2019). This complex effect of the protein origin and carbohydrates structure and rumen conditions does not just affect the productivity indices, such as milk yield and feed efficiency but also the production of milk components, such as different fatty acid profiles (Stork et al., 2024). It proves that certain food ingredients may adjust the nutrient use and minimize its effects on the environment by changing the protein avoidance mechanism and lowering the level of methane emissions (Cherdthong, 2024; Guevara et al., 2024). In particular, the insect food supplement has been found to minimize the methane production, which may be explained by the increased content of fat in this food, which has been proved to reduce the mechanism of methane production in animals (Cherdthong, 2024). It shows that alternative forms of protein can help transform the dairy farming into a more eco-friendly system that would not only maximize the efficacy of farm foods but also would have a lesser environmental footprint of cattle at the same time (Wesemael et al., 2018). Additional research finds that the wholly replaced soybean meal in dairy cows concentrate with protein sources that are derived in yeast like *Candida*

*jadinii* can be non-relevant to the rumen microbiota, fatty acid composition of the milk, or sensory qualities of milk (Stork et al., 2024). This would be an opportunity to make the regions more autonomous concerning the imported protein since global food systems are prone to the shocks caused by politics and climate change (Stork et al., 2024). It can also be explained by the fact that fatty acid structure of milk does not change significantly when yeast replaces soybean meal with the difference in the amount of the total daily dry intake of matter and the level of fatty acids in the concentrate (Stork et al., 2024). The findings are incredibly vital in designing viable dairy production systems that reduce reliance on imported feedstuffs at the cost of milk quality and milk quantity (Stork et al., 2024). In addition, the fatty acid profiles of new protein sources are likely to be altered, and approximately half of the lipids in milk are produced in vivo, with the remainder of the lipid components being provided by the diet, although rumen biohydrogenation has a significant influence on them (Stork et al., 2024). This shows that the relationship between the ruminal biohydrogenation and milk fat and the fatty acid makeup of the food was complex. It also indicates the significance of making a prudent choice on the various sources of proteins in order to attain the required milk quality properties. The fact that the innovative sources of proteins (rapeseed cake and yeast-resin) may enhance the utilization of nitrogen and milk yield, as well as, simultaneously, reduce the level of saturated fatty acids in the milk fat, only augments the multi-fold benefits of feed formula diversification (Wang et al., 2022). With their addition, too, of these other alternative proteins, such as detoxified jatropha meal and other single-cell proteins (microalgae and duckweed) it can also be raised in large scale without arable land and good weather, which further makes the process more sustainable (Wesemael et al., 2018). A yeast microbial protein, which has a source of *Cyberlindnera jadinii*, is one such substance that can potentially replace soybean meal in barley-based concentrates without modifying the fatty acid profile of the milk, the rumen microbiota,



and milk taste (Stork et al., 2024). This choice is especially attractive to the cheese production process because it was shown that cow milk may have a higher casein content when it is being fed a food high in yeast, and it can affect the quality of cheese production (Pedersen et al., 2022). It implies that the yeast protein can be used as a decent alternative to the conventional sources of proteins other than adding some added value to the manufacture of dairy products (Olsen et al., 2023; Stork et al., 2024). Moreover, the other opportunities of sustainable feed plans have future-prospects to substitute the common soybean protein with industrial waste products, including NexPro in the dry-mill bioethanol process, into the dairy cow feed system without affecting the milk production and its makeup (Pedersen et al., 2022). Such development is extremely important in order to make agricultural systems more circular or minimize the rivalry between food sources that people and animals hold. Other scientists have documented a dramatic decrease in ruminal methane production and a shift in the fatty acid composition of milk, a decrease in the ratio of saturated acids and an increase in the ratio of cis-9 18: 1 with no effect on the organoleptic properties of milk (Halmemies-Beauchet-Filleau et al., 2023).

### Conclusion

The presented research provides ample evidence to indicate that substitutes of normal soybean meal by agro-industrial by-products, old foodstuffs, and distillers grains can effectively replace the regular soybean meal in the dairy cow diets without reducing its productive performance. The cows that were provided with non-protein diets maintained or enhanced their milk production, dietary efficiency, and digestible nutrients. They were also more efficient in nitrogen and were less evident of environmental loss. The fact that nitrogen was more likely to be found in milk protein rather than in urine and feces indicates that such feeding activities may be used to decrease nitrogen pollution and make dairy production more environment-friendly. Moreover, microbes were more productive with

good rumen fermentation patterns when using the various types of protein and the patterns of fermentation remained constant and it was indicated that the amount of enteric generated methane would be reduced. Financially, the income was consistently larger than the feed expenses with the inclusion of cheaper protein substitutes and this demonstrates that the business was very stable even during volatile feed prices. Notably, the utilization of protein sources that cannot be directly consumed by human beings can aid in the rising competition between food and feed as well as making dairy production safer and more sustainable throughout the world. In the case of precision feeding systems, such alternative protein methods could be applied in order to maximize production, profits and environmental preservation simultaneously. Altogether, this paper shows that the concept of strategic replacement of traditional sources of proteins with more sustainable ones is a practical and progressive solution to the challenges of the current dairy systems, which facilitate the sustainability of the farms in the long-term and the decrease in the ecologic footprint of the milk production.

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