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## The physiological and metabolic health responses of the Holstein cows to divergent energy sourcing strategies in semiarid environments

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### Abstract

The challenge of ensuring metabolic homeostasis and maximizing lactational performance is of vital concern in the modern dairy industry, especially in high-yielding Holstein-Friesian cows in semi-arid conditions. This experiment was based on the peri-urban areas of South Baghdad, Iraq, to offer a detailed physiological examination and consider the systemic health signs in cows as divergent energy sourcing strategies respondents. This was done to determine nutritional interventions that could favor productivity without causing an increase in metabolic stress or the loss of physiological integrity. A total of 20 cows (3-4 years old) were selected and underwent an intensive 60-day period of metabolic stabilization in a basal Total Mixed Ration (TMR) to stabilize pre-experimental health status. Animals were then placed in four diets, which included Control, Saturated Fat, Bypass Fat, and Glycerol. There were also 345 valid individual records produced and analyzed with a Mixed Model for Repeated Measures (MMRM) to consider individual variability and temporal changes in health. The findings showed that Bypass Fat supplementation maximized productive efficiency by recording the highest daily milk production ( $37.1 \pm 2.9$  kg/day) as opposed to the Control ( $33.6 \pm 2.4$  kg/day) group ( $P < 0.05$ ). Health-wise, Bypass Fat enhanced milk protein concentration ( $3.03 \pm 0.15$ ) and did not change dietary fiber interactions ( $34.0 \pm 1.0$ ), indicating that rumen-protected lipids do not affect ruminal fermentation or cause subclinical metabolic disorders. Glycerol supplementation, on the other hand, had a significant decrease in the milk yield ( $12.1 \pm 1.4$  kg/day) but the highest protein ( $3.28 \pm 0.17\%$ ) and fat ( $3.02 \pm 0.22\%$ ) levels. This means that we have metabolic plasticity, but the yield depression implies that there are changes in hepatic gluconeogenesis and nutrient partitioning that might not be ideal in the long-term systemic health in heat-stressed areas. We find that Bypass Fat offers a better physiological and health balance, which protects the metabolic condition of the herd, and maximum productivity is obtained. The findings recommend the incorporation of rumen-protected lipids to improve the economic productivity and the health of dairy cows in arid agro-ecosystems that are driven by welfare, Semiarid Environments

**Keywords:** Bypass fat, Holstein-Friesian cows, milk composition, physiological assessment, systemic health

### Introduction

The major goal in the modern dairy business is to maximize the lactational performance and maintain the metabolic homeostasis and physiological well-being of high-producing animals. The Holstein-Friesian cows with high genetic milking capacity are especially vulnerable to physiological stressors when they are kept in the demanding agro-ecological areas like the semi-arid regions of Central Iraq. In the early and middle of lactation, such cows tend to experience a Negative Energy Balance (NEB), a metabolic condition in which the energy needed to produce and maintain milk is greater than the amount of calories consumed in the diet (Coleman *et al.*, 2022). Unless addressed efficiently with the help of the specific nutritional treatment, NEB may initiate a cycle of metabolism disorders, such as ketosis and fatty liver, which can severely disrupt the overall health and productive efficiency in the long run (Loor *et al.*, 2021; Wang *et al.*, 2024) [3, 7, 14].

The physiological capacity of dairy herds to heat stress, such as those found in South Baghdad, requires the formulation of dietary energy-intensive solutions that do not impair the ruminal

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fermentation and systemic oxidative homeostasis (Salami *et al.*, 2023). Historically, the grain-to-forage ratio was the most common approach to boosting energy density, but it is associated with subacute ruminal acidosis (SARA), which has a negative impact on the health condition of a living being and the digestibility of fibers (Meale *et al.*, 2021). This has led to the development of scientifically viable options to promote metabolic stability by the use of divergent energy-sourcing strategies, namely rumen-protected lipids and glucogenic precursors (Daneshi *et al.*, 2024; Kashiri *et al.*, 2024) [12, 10, 4, 6].

Calcium salts of fatty acids (bypass fats) are rumen-protected fats, which are a concentrated source of energy, and they do not undergo the microbial fermentation process in the rumen but are absorbed directly into the small intestine (Vargas-Bello-Pérez *et al.*, 2021). Healthwise, this is important since it avoids the antimicrobial actions of unsaturated fatty acids on cellulolytic bacteria, which ensures maximum ruminal motility and degradation of fiber (Castillo-Lopez *et al.*, 2021). Moreover, bypass fats are found to positively affect nutrient allocation and optimize the biochemical composition of dairy cows by decreasing the use of body fat mobilization, which protects the health of the liver (Martínez *et al.*, 2023; Pascotto *et al.*, 2022) [13, 2, 9, 11].

Simultaneously, glucogenic nutritional supplements, including glycerol, were explored as having the capacity to directly affect hepatic gluconeogenesis and blood glucose (Arshad *et al.*, 2020). Glycerol is an active precursor that can regulate the release of insulin and change the distribution of nutrients to mammary protein production (Kashiri *et al.*, 2024). Nevertheless, the glycerol action on the body is complicated and may differ significantly depending on the environmental conditions and the initial metabolic state of the animal (Meale *et al.*, 2021). Although the latter points out its advantages in minimizing the risk of ketosis, some studies indicate that elevated concentrations of glucogenic precursors could redirect the energy not to milk volume, which is a distinctive feature of metabolic plasticity (Zhang *et al.*, 2025). Learning the interplay between these energy strategies and the dietary fiber (NDF) profile is key to the maintenance of the physiological health of the cow. The factor that determines the entire systemic health is the maintenance of an optimal ruminal environment because it affects the inflammatory response, up to the fact that the animal can respond to the environmental heat loads (Gandra *et al.*, 2023). Although abundant data on the topic is available globally, there is a severe dearth in the literature that measures the relative physiological and health effects of these energy-sourcing strategies in the particular agro-climatic environment of Iraq. In the majority of local research, much of the attention was paid to simple production measures, and the metabolic indicators underlying the long-term viability of the herd were overlooked (Mahmoud *et al.*, 2022) [1, 6, 10, 15, 5, 8].

The purpose of the study was, therefore, to offer a holistic physiological analysis and measurement of the systemic health indicators of Holstein-Friesian cows to the divergent energy sourcing strategies in South Baghdad. This study will be able to determine nutritional pathways that maximize productive efficiency and promote the metabolic well-being and physiological stability of high-yielding dairy cows fed in arid conditions by including a detailed analysis of milk yield, milk composition, and dietary interactions.

## Materials and methods

### Ethical Approval and Experimental Site

The experimental procedures were carefully performed in line with the ethical provisions of animal welfare and scientific research. The experiment was carried out in the agricultural peri-urban agricultural belt of South Baghdad, Iraq, which is a semi-arid area with varying environmental stressors. The fieldwork part of the study was conducted during the fall of the research, that is, in September, October, and November 2025, to assess the response of the animals in the period of climatic transition in Central Iraq.

### Homogenization Protocol and Animal Selection

A group of twenty multiparous Holstein-Friesian cows (n=20) was carefully chosen among a bigger herd on the basis of standardized criteria in order to guarantee physiological and metabolic uniformity. The total number of cows chosen was 3 to 4 years old and had a reproductive history that was consistent and had two calvings (parity = 2) only. A strict metabolic adaptation period of 60 days was conducted before the trial in order to remove carry-over effects and stabilize the baseline metabolic condition. In this period, all animals were kept at a constant mix of Total Mixed Ration (TMR) so that the ruminal fermentation trends and systemic health indicators could be equilibrated.

### Experimental Energetic Strategies and Diets

The cows were then fed under four different strategies of sourcing dietary energy following the stabilization phase. The basal diet was developed as a TMR, and the experimentation made the following changes:

- **Control Group:** Was given the basal TMR without exogenous lipid or glucogenic supplementation.
- **Saturated Fat Group:** Supplemented with a palmitic acid-rich fat (around 23 percent of DM) to act as a source of direct saturated lipids.
- **Bypass Fat Group:** Rumen-protected lipids as calcium salts of fatty acids (CSFA) 2-3% DM. This was aimed at supplying a ruminal inert source of energy that will not be degraded by microbes.
- **Glycerol Group:** Added a pure glucogenic energy source (Glycerol) to determine the effect of direct glucose precursors on the nutrient partitioning and milk solids synthesis.
- **Milking and Data Collection Processes.**
- The major data was obtained based on 345 valid records of individuals used in the three months of the experiment.
- **Milk Production:** Milk yield was observed and measured separately for each cow using electronic scales that were calibrated. Milking was performed twice daily at 07:00 h and 17:00 h. The arithmetic addition of the morning and evening sessions to the daily milk yield (kg/day) was used.
- **Sample Collection:** Composite milk samples were collected by mixing up morning and evening aliquots proportionally to the yield of each cow in the morning and evening. In the case of dietary analysis, the TMR samples were taken on a weekly or 10-14-day basis in order to keep a check on the consistency of the Neutral Detergent Fiber (NDF) as well as the other nutrient fractions.

### Specialized Analysis of Laboratory

MilkoScan FT+ (or FT120)- Fourier Transform Infrared Spectroscopy (FTIR)-based analytical platform was used to analyze fat percentages and protein percentages in milk. This instrumentation offered very accurate molecular profiling of

milk components, which is a surrogate of the metabolic health condition of the animal.

**Feed and Fiber Analysis:** Feed analysis of the feed was done via the Van Soest detergent system to determine the NDF content of the feed. The samples were first dried in a special oven, a laboratory mill with a 1 mm screen was used, and then analyzed by a Fiber Analyzer (ANKOM or similar) to guarantee the accurate determination of the structural carbohydrate fraction.

**Statistical Modeling:** Mixed Model of Repeated Measures (MMRM) analysis was done on the data. The statistical model included dietary treatment as a fixed effect, whereas the individual cow was included as a random effect in order to consider inherent physiological variation. As a repeated factor, time (day/week in the 3-month period) was incorporated. In order to achieve the maximum level of statistical rigor, a number of covariance structures (AR1 and Compound Symmetry) were tested, and the most parsimonious model was chosen, using the Akaike Information Criterion (AIC). Residual diagnostics were done to verify the assumptions of normality and homoscedasticity. The Tukey-Kramer adjustment was used to do post-hoc mean separations with  $P > 0.05$  as the level of statistical significance.

**Results:** Table 1 and Figure 1 provide the results of a comparative analysis of the effect of divergent energy sourcing strategies on milk production and composition. The dietary treatments had a significant impact on all the parameters that were measured ( $P < 0.05$ ), proving that Holstein-Friesian cows were very sensitive to the metabolic character of lipid and glucogenic supplements within the South Baghdad environment.

#### Volumetric Yield and Lactational Performance

The energy diet plan had a far-reaching effect on the everyday milk production. The Bypass Fat group (calcium salts of fatty acids) had the best statistical and numerical value of  $37.1 \pm 2.9$  kg/day. This is a great improvement as compared to the Control group ( $33.6 \pm 2.4$  kg/day) and was better than the Saturated Fat treatment ( $35.9 \pm 2.7$  kg/day). On the other hand, the Glycerol Supplementation group had a significant decrease in volumetric output, with the lowest average amounting to  $12.1 \pm 1.4$  kg/day, and this is a strong indication of a change in nutrient partitioning towards not producing fluid milk.

#### Fat and Protein Milk Compositional Profiling

Even though the total volume decreased, the Glycerol group had the highest Glycerol group with a milk protein content of  $3.28 \pm 0.17$  percent and fat content at  $3.02 \pm 0.22\%$ . Interestingly, there was also a significant change in the milk protein concentration ( $3.03 \pm 0.15\%$ ) in the Bypass Fat group when compared to both the Control ( $2.90 \pm 0.12\%$ ) and Saturated Fat group ( $2.89 \pm 0.14\%$ ). The percentages of milk fats were relatively constant across the lipid-supplemented treatments, with Saturated Fat treatment generating ( $2.91 \pm 0.21\%$ ) and Bypass Fat treatment generating ( $2.87 \pm 0.19\%$ ), as indicated in Figure 2.

#### Rumen Health Indicators and Dietary Fiber (NDF)

Figure 3 indicates that the Neutral Detergent Fiber (NDF) content of the Total Mixed Ration (TMR) was followed as an

indicator of ruminal stability. The NDF levels were uniform in all the lipid treatments, with the Control group showing 32.8% DM and the Bypass Fat group showing 34.0% DM. In the case of the Glycerol treatment, the NDF value was kept at 31.5% DM, which is the basal diet NDF as indicated in the experiment parameters. These results indicate that the addition of rumen-protected lipids (Bypass Fat) had no adverse effect on the fiber content of the diet.

#### Discussion

The metabolic and physiological processes, which were observed in this study, offer imperative information regarding the adaptive ability of the Holstein-Friesian cows that were kept under the semi-arid environment of Central Iraq. The high sensitivity of the mammary gland and systemic metabolism to the biochemical character of supplemental energy sources is indicated by the great differences in lactational performance and milk compositions among the four dietary treatments. Management of Negative Energy Balance (NEB), a condition that not only reduces production but also endangers the metabolic health and physiological homeostasis of the dairy cows, is the greatest challenge in such environments (Coleman *et al.*, 2022; Loor *et al.*, 2021)<sup>[3, 7]</sup>.

#### Physiological Superiority of Bypass Fat in the Partitioning of Energy:

The most vivid outcome of the study was the great maximization of the milk yield of the Bypass Fat group ( $37.1 \pm 2.9$  kg/day). Mainly, it is a physiological betterment of the rumen-inert calcium salts of fatty acids (CSFA). Contrary to unprotected or saturated fats, bypass fats do not undergo microbial fermentation in the rumen, and as such, they do not undergo the biohydrogenation process that frequently results in the formation of trans-fatty acid isomers that inhibit milk fat production (Castillo-Lopez *et al.*, 2021; Vargas-Bello-Pérez *et al.*, 2021). These lipids are also directly absorbed in the small intestine by bypassing the rumen, which supplies a concentrated stream of energy to counter the thermic effect of feeding, which is a critical factor in maintaining health in the heat-stressed environment of South Baghdad (Salami *et al.*, 2023)<sup>[2, 12, 13]</sup>.

Moreover, the fact that NDF levels ( $34.0 \pm 1.0\%$ ) in the bypass fat group were stable implies that this active approach did not cause any damage to the ruminal microbiome. The lack of antimicrobial impact on cellulolytic bacteria will guarantee the proper digestion of fibers and ruminal pH, which is the keystone to the systemic health of ruminants (Wang *et al.*, 2024). The noted growth of the milk protein ( $3.03 \pm 0.15$ ) also indicates the presence of a so-called glucogenic-sparing effect, in which the higher the availability of dietary lipids to oxidize, the less the dietary amino acids are required to hepatic gluconeogenesis, and the more amino acids are directed towards the synthesis of mammary proteins (Martínez *et al.*, 2023; Zhang *et al.*, 2025). The presented change in nutrient proportions is one of the major indicators of a better metabolic rate and physiological strength (Gandra *et al.*, 2023)<sup>[14, 9, 15, 5]</sup>.

#### Glycerol Metabolic Plasticity and the Glucogenic Effect of Glycerol:

The reaction of the Glycerol group showed a peculiar aspect of metabolic plasticity. Although the volumetric milk yield was significantly decreased ( $12.1 \pm 1.4$  kg/day), the drastic increase in the percentage of milk protein ( $3.28 \pm 0.17\%$ ) and fat ( $3.02 \pm 0.22\%$ ) indicates that there was a massive change in the systemic nutrient partitioning.

Glycerol is an effective glucogenic precursor, and it is rapidly absorbed through the ruminal epithelium or converted to propionate, which prompts the hepatic gluconeogenesis and eventual insulin release (Arshad *et al.*, 2020; Kashiri *et al.*, 2024). The elevation of systemic insulin stimulates the absorption of amino acids and glucose into the tissues of the body and has the potential to redistribute energy at the expense of the mammary gland producing fluid milk, particularly when the prevailing metabolic state of the animal is not matched with high-volume production (Meale *et al.*, 2021) [1, 6, 10].

Healthwise, although glycerol is commonly employed in the prevention of subclinical ketosis through direct supply of glucose, its long-term effects on high-yielding cows in heat-stressed areas must be managed with the help of caution. The observed extreme yield reduction could reflect a metabolic compromise in which the cow could be more focused on the concentrated nutrient output rather than the volume to ensure physiological stability in a stressful environment (Daneshi *et al.*, 2024). This implies that although glycerol is a promising option in terms of enhancing day-neutral milk production or transitional milk-based health options, it might not be the main approach to optimizing daily output in dry agro-ecosystems, as rumen-protected lipids (Mahmoud *et al.*, 2022) [4, 8].

**Whole-Body Health, Oxidative Balance, and Focal Adaptability:** The significance of dietary heat increment management is demonstrated by the fact that bypass fats are already integrated in the autumn season in Iraq (September–November 2025). Saturated and bypass fats also have the advantage of a lower heat increment than the fermentation of the structural carbohydrates (NDF), which lowers the heat load to the internal metabolism of the animal (Pascotto *et al.*, 2022). This decrease in thermic stress is necessary to ensure systemic oxidative balance and avert the inflammatory response that may frequently follow the joint effect of elevated production and elevated ambient temperatures (Salami *et al.*, 2023).

Besides, the protection of hepatic health is an important aspect of the metabolic health status. Bypass fats decrease the release of non-esterified fatty acids (NEFA) into the bloodstream by adipose tissue, which contributes to the occurrence of hepatic lipidosis (fatty liver) and preserves the liver adenosine triphosphate (ATP) required to perform essential metabolic processes (Martínez *et al.*, 2023; Wang *et al.*, 2024). Such extensive protection of the physiological well-being of the cow is what guarantees that the cow is able to maintain the highest productivity (37.1 kg/day) without reducing its long-term welfare or reproduction potential (Loor *et al.*, 2021) [11, 12, 9].

**Table 1:** Effect of Divergent Energy Sourcing Strategies on Productivity and Metabolic Indicators (Mean  $\pm$  SD)

| Diet Type / Treatment | NDF (% DM)      | Milk Yield (kg/day) | Milk Fat (%)    | Milk Protein (%) |
|-----------------------|-----------------|---------------------|-----------------|------------------|
| Control TMR           | 32.8 $\pm$ 1.1  | 33.6 $\pm$ 2.4      | 2.88 $\pm$ 0.18 | 2.90 $\pm$ 0.12  |
| Saturated Fat         | 33.4 $\pm$ 0.9  | 35.9 $\pm$ 2.7      | 2.91 $\pm$ 0.21 | 2.89 $\pm$ 0.14  |
| Bypass Fat            | 34.0 $\pm$ 1.0  | 37.1 $\pm$ 2.9a     | 2.87 $\pm$ 0.19 | 3.03 $\pm$ 0.15b |
| Glycerol              | 31.5 $\pm$ 1.3* | 12.1 $\pm$ 1.4      | 3.02 $\pm$ 0.22 | 2.98 $\pm$ 0.17  |

\*NDF represents the basal diet value as specified in the source. <sup>a,b</sup> Indicates significant differences compared to the Control ( $P < 0.05$ ).

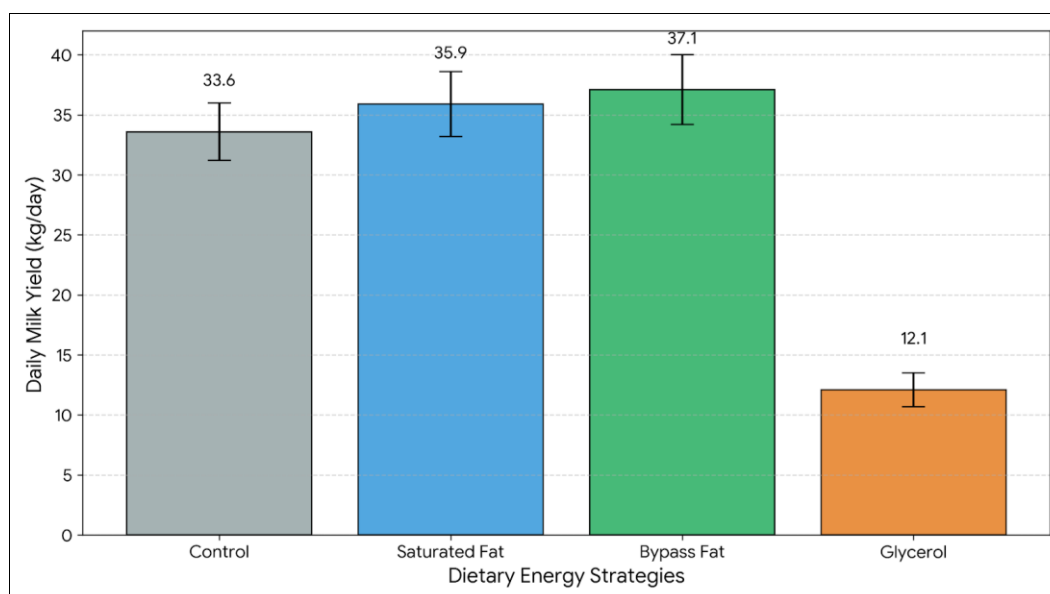


**Fig 1:** Comparison of Daily Milk Yield Across Energy Supplementation Groups

Figure 1 demonstrates the relative effect of divergent energy sourcing strategies on the daily milk yield of the Holstein-Friesian cows. The results indicate clearly that Bypass Fat supplementation (calcium salts of fatty acids) was most effective with the highest physiological output of 37.1  $\pm$  2.9 kg/day, which was much higher than that of the Control group (33.6  $\pm$  2.4 kg/day) and the Saturated Fat group (35.9  $\pm$  2.7

kg/day). Conversely, the Glycerol group had a significant decrease in volumetric yield (12.1  $\pm$  1.4 kg/day). This graphic demonstrates the metabolic efficiency of rumen-protected lipids in sustaining high lactational demands and the unique nutrient partitioning impact of glucogenic precursors of the South Baghdad environment.

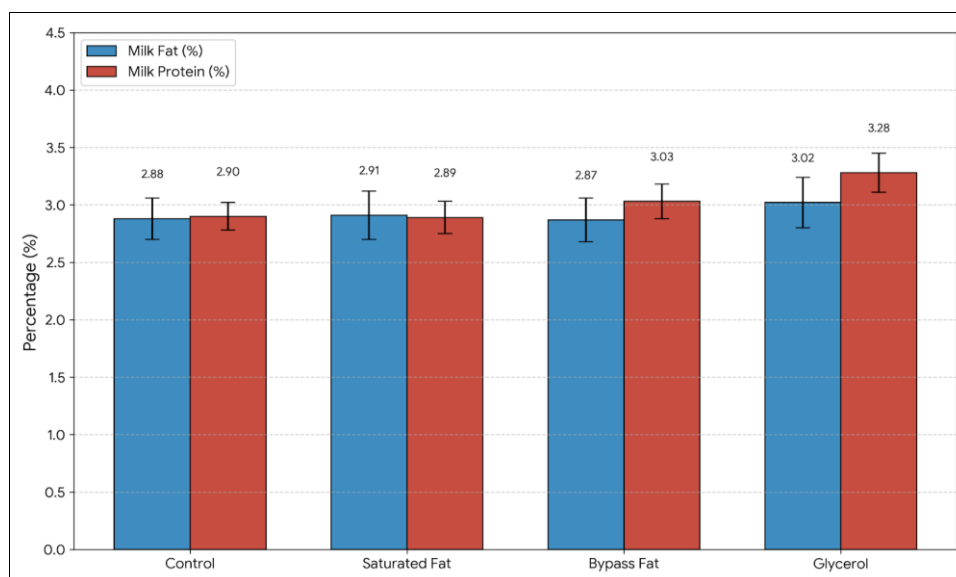


**Fig 2: Comparative Analysis of Milk Composition (Fat and Protein%)**

Figure 2 offers a comparative study of the milk fat and protein content in the different divergent dietary treatments. The results indicate the high milk protein content in the Glycerol ( $3.28 \pm 0.17\%$ ) and Bypass Fat ( $3.03 \pm 0.15\%$ ) is significantly higher than in the Control ( $2.90 \pm 0.126$ ). Milk fat was relatively constant in the lipid-supplemented groups, with the

highest concentration being in the Glycerol treatment ( $3.02 \pm 0.22\%$ ). These findings graphically affirm the metabolic plasticity of the Holstein-Friesian cows, where alternative energy sources have been successful in regulating nutrient allocation and mammary synthesis of milk solids.

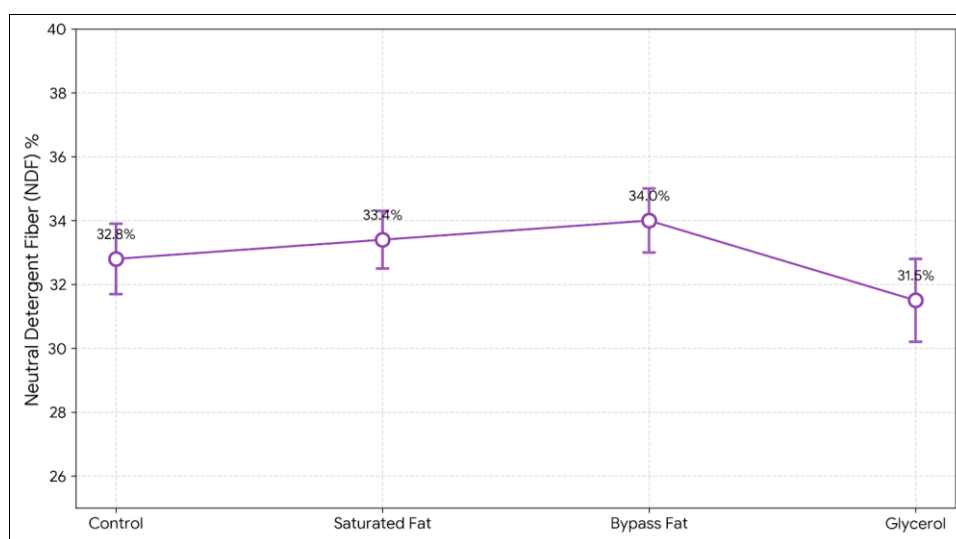


**Fig 3: Stability of Dietary Fiber (NDF) Across Treatment Groups**

Figure 3 indicates the consistency of the Neutral Detergent Fiber (NDF) fraction in the Total Mixed Ration (TMR) in all the experimental groups. The results show that the NDF levels were physiologically the same ( $34.0 \pm 1.0$  vs.  $32.8 \pm 1.1$ ) between the Bypass Fat group, which is high in energy density, and the Control. This trend graphically confirms that rumen-protected lipids used in this research did not affect dietary fiber structure or ruminal fermentation environment, which is an important indicator of the remaining systemic wellness and maximum microbial activity.

## Conclusion

To sum it up, this research paper shows that the strategic incorporation of Bypass Fat (calcium salts of fatty acids) is the most physiologically viable energy-sourcing policy of Holstein-Friesian cows reared in a semi-arid climate of South Baghdad. The practice was effective in increasing the volume

of milk production and enhancing the protein level, as well as protecting the metabolic well-being and ruminal performance of the herd. Although glycerol supplementation is an important source of the glucogenic pathway in increasing milk solids, the effects on total yield indicate a metabolic partitioning that is not the best under heat stress to produce high-volume milk. Thus, the use of rumen-protected lipids as a major nutritional intervention in order to maximize the economic performance and systemic conditions of dairy cows in arid lands is advised.

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### Conflicts of interest

The authors declare no conflict of interest.

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