



## Ameliorative Effects of *Moringa oleifera* Supplementation on Metabolic, Oxidative, and Reproductive Alterations Induced by Feed Restriction in Female Rats

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### ABSTRACT

Nutritional stress such as feed restriction can negatively affect metabolic balance, oxidative status, and reproductive function, particularly in females. *Moringa oleifera* is a natural supplement rich in antioxidants and bioactive compounds that may help counteract such disruptions. This study aimed to evaluate the potential protective effects of *Moringa oleifera* supplementation on metabolic, oxidative, and reproductive parameters in adult female rats subjected to normal or restricted dietary intake. Twenty-four adult female rats were randomly divided into four groups (n = 6 per group): a control group (standard diet), a standard diet + *Moringa* (500 mg/kg BW), a 30% feed restriction, and 30% feed restriction + *Moringa*. The experimental period lasted four weeks. Body weight was recorded weekly. At the end of the study, blood samples were analyzed for fasting plasma glucose, lipid profile (cholesterol, triglycerides), and oxidative stress markers (total antioxidant capacity [TAC] and malondialdehyde [MDA]). Vaginal smears were used to assess estrous cycle phases and corresponding levels of reproductive hormones (estradiol and progesterone). Feed restriction significantly decreased body weight, TAC, estradiol, progesterone, glucose, cholesterol, and triglyceride levels, and significantly increased MDA levels compared to the control group. In contrast, *Moringa oleifera* supplementation, either alone or combined with feed restriction, significantly improved body weight, normalized glucose and lipid profiles, enhanced TAC, reduced MDA, and restored estradiol and progesterone levels. *Moringa oleifera* supplementation effectively counteracted the physiological disturbances induced by feed restriction in female rats. These findings suggest its potential as a supportive dietary strategy to protect reproductive and metabolic health during periods of nutritional stress.

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### INTRODUCTION

Feed remains the most significant input cost in animal production, and both the quality and quantity of dietary intake play critical roles in sustaining metabolic balance, reproductive performance, and overall animal health (Pollesel *et al.*, 2020; Cavallini *et al.*, 2023). Nutritional restriction, whether in quantity or nutrient density, has been linked to a range of reproductive dysfunctions, including suppressed gonadotropin release, impaired folliculogenesis, delayed puberty, and extended postpartum anestrus (Guo *et al.*, 2018; Iwasa *et al.*, 2018). These disruptions are often more pronounced in female animals, where maternal undernutrition not only affects the dam but also

compromises fetal growth, placental function, and long-term offspring health outcomes (Nitzsche, 2017).

Moreover, feed restriction is associated with elevated oxidative stress, which can damage reproductive tissues and interfere with cellular signaling, increasing the risk of apoptosis and endocrine disruption (Walsh *et al.*, 2014; El-Sherbiny *et al.*, 2024). In this context, nutraceutical interventions using plant-derived compounds have gained attention for their potential to mitigate stress-induced damage. Among these, *Moringa oleifera*, a nutrient-dense tree native to South Asia and Africa, has emerged as a promising natural feed additive due to its high content of vitamins (A, C, and E), minerals, flavonoids, polyphenols, and

bioactive peptides (Maher *et al.*, 2023; Nurhayati *et al.*, 2023).

Recent studies have demonstrated that *Moringa oleifera* exerts protective effects on metabolic and reproductive functions, especially under conditions of nutritional or oxidative challenge. These effects are largely attributed to its modulation of key physiological pathways, including activation of the Nrf2/ARE pathway involved in antioxidant defense, AMPK/ACC signaling relevant to lipid and energy metabolism, and regulation of the hypothalamic–pituitary–gonadal (HPG) axis, which governs reproductive hormone release (Iwasa *et al.*, 2018; Hong *et al.*, 2023).

Despite its recognized benefits, there remains a lack of data regarding the capacity of *Moringa oleifera* to counteract the metabolic, oxidative, and hormonal disturbances induced by feed restriction, particularly in female models. Given the dynamic and hormonally sensitive nature of the estrous cycle, it is crucial to investigate whether *Moringa oleifera* supplementation can restore physiological stability during periods of dietary stress.

This study aims to address this gap by evaluating the protective role of *Moringa oleifera* against feed restriction-induced alterations in body weight, oxidative stress, glucose and lipid metabolism, and reproductive hormone levels in adult female rats.

## MATERIALS AND METHODS

### Ethical Approval and Animal Care

The present study was conducted on 24 adult female Swiss albino rats (150–180 g), obtained from the Medical Entomology Research Institute, Giza, Egypt. All experimental procedures were carried out at the Department of Physiology, Faculty of Veterinary Medicine, Cairo University. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Cairo University (Approval No. Vet CU110520251093), in accordance with the guidelines of the National Institutes of Health (NIH, 2011) for the ethical care and use of laboratory animals.

Upon arrival, the animals were acclimatized for two weeks under controlled environmental conditions, including a 12-hour light/dark cycle, ambient temperature of 23–25°C, and relative humidity of 60–65%. Rats were housed in polypropylene cages (35.60 × 63.55 × 24.68 cm) with galvanized iron filter tops. A standard pelleted diet containing 18% crude protein, 86% dry matter, 5.5% crude fiber, and 2.5% crude fat, along with essential vitamins and minerals, was provided. Water was available *ad libitum*.

### Experimental design

The animals were randomly divided into four groups (n = 6 per group) and treated for four weeks as follows:

**Group I (Control):** Received 100% of the standard diet without supplementation.

**Group II (Moringa):** Received 100% of the standard diet supplemented with *Moringa oleifera* leaf powder via oral gavage at a fixed dose (500 mg/kg BW/day).

**Group III (Restricted):** Received 70% of the standard diet (30% feed-restricted) with no supplementation (Nitzsche, 2017).

**Group IV (Restricted + Moringa):** Received 70% of the standard diet supplemented with *Moringa oleifera* (500 mg/kg body weight).

The dosage and nutritional profile of *Moringa oleifera* leaf powder were based on previously published in studies (Nurhayati *et al.*, 2023). The *Moringa* leaf powder is dissolved with the same volume of water (2 mL) for each group. Supplementation was administered daily via oral gavage throughout the experimental period. The *Moringa oleifera* leaf powder was purchased from a certified herbal supplier in Egypt: Haraz for Herbs & Spices, Giza, Egypt.

**Table 1:** Chemical composition of *Moringa oleifera* leaves (MOL) on a dry matter basis according to (Zeng *et al.*, 2019).

Item	Content
Crude protein (g/kg)	270.4
Crude fat (g/kg)	74.3
Crude fiber (g/kg)	29.5
Ash (g/kg)	79.6
Calcium (g/kg)	15.8
Total phosphorus (g/kg)	6.1
Iron (Fe) (mg/kg)	202.3
Potassium (K) (g/kg)	17.9
Magnesium (Mg) (g/kg)	4.6
Lysine (g/kg)	13.5
Methionine (g/kg)	2.2
Vitamin E (g/kg)	0.5

### **Body weight measurement**

Body weights were recorded on a weekly basis. To minimize variability due to recent food intake, feed and water were withheld 12 hours prior to weighing.

### **Sample collection**

At the end of the 4-week treatment period, animals were fasted overnight. Anaesthesia was induced by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Blood samples were collected from the retro-orbital sinus using heparinized microcapillary tubes. Serum Preparation: Blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes at 4°C. The resulting serum was aliquoted and stored at -20°C for subsequent biochemical and hormonal analyses. Plasma Glucose: For plasma glucose determination, blood was collected in fluoride-containing tubes to inhibit glycolysis.

### **Oxidative stress biomarkers**

Markers of oxidative stress were evaluated using commercially available kits (Bio-Diagnostic, Cairo, Egypt). Total Antioxidant Capacity (TAC): Assessed according to the method of **Koracevic *et al.*, (2001)** (Cat. No.: TA 2512). Malondialdehyde (MDA): Measured using the TBARS method as an indicator of lipid peroxidation (**Ohkawa *et al.*, 1979**) (Cat. No.: MD 2539).

### **Hormonal analysis**

Serum concentrations of estradiol and progesterone were measured during the estrous cycle using a double-antibody sandwich ELISA technique. Assays were performed according to the manufacturer's instructions using commercial kits (LEADER TRADE, Cairo, Egypt), and analyzed with an ELISA microplate reader. Estradiol (E2): ELISA Kit (Cat. No.: LE-2202). Progesterone (P4): ELISA Kit (Cat. No.: LE-2104).

### **Statistical analysis**

Data are expressed as mean  $\pm$  standard error of the mean (SEM). Statistical differences among the experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Analyses were performed using SPSS software (Version 20.0, IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant. Graphs were generated using GraphPad software (version 6).

### **Vaginal cytology**

Vaginal cytology was performed daily throughout the experimental period to assess the stages of the estrous cycle. The pipette smear technique was employed (**Marcondes *et al.*, 2002**), in which a drop of sterile saline was introduced into the vaginal orifice, aspirated, and transferred to a glass slide. The slides were examined under light microscopy for cytological evaluation.

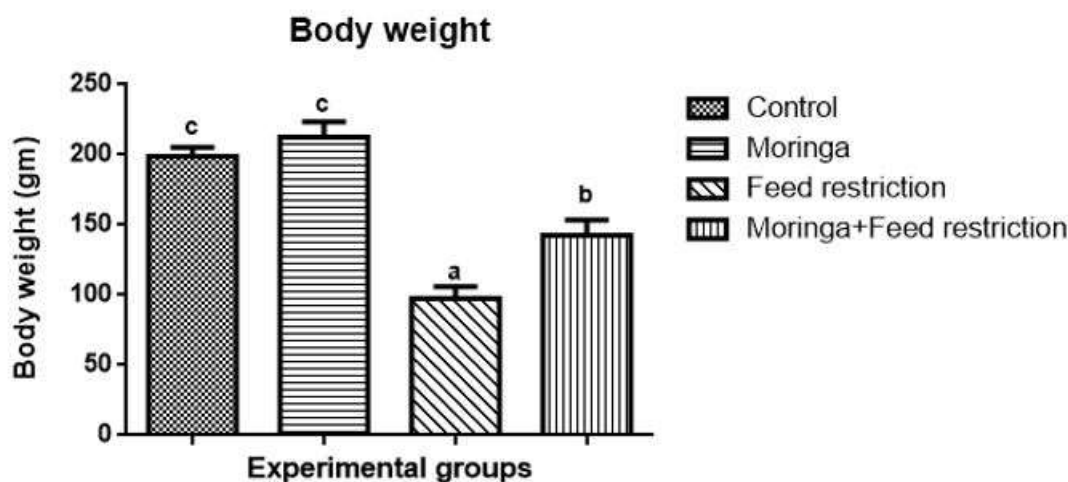
### **Biochemical analysis**

Biochemical parameters were determined using standard colorimetric methods with commercial kits (Spectrum Diagnostics, Cairo, Egypt) and a UV-visible spectrophotometer (Jasco V-730, Japan). Plasma Glucose: Measured using the glucose oxidase method (**Trinder, 1969**) (Cat. No.: 230011). Total Cholesterol: Estimated as described by **Young *et al.*, (1975)** (Cat. No.: 310002). Triglycerides: Quantified using the method reported by **Vassault *et al.*, (1999)** (Cat. No.: 330005).

## **RESULTS**

### **Body weight**

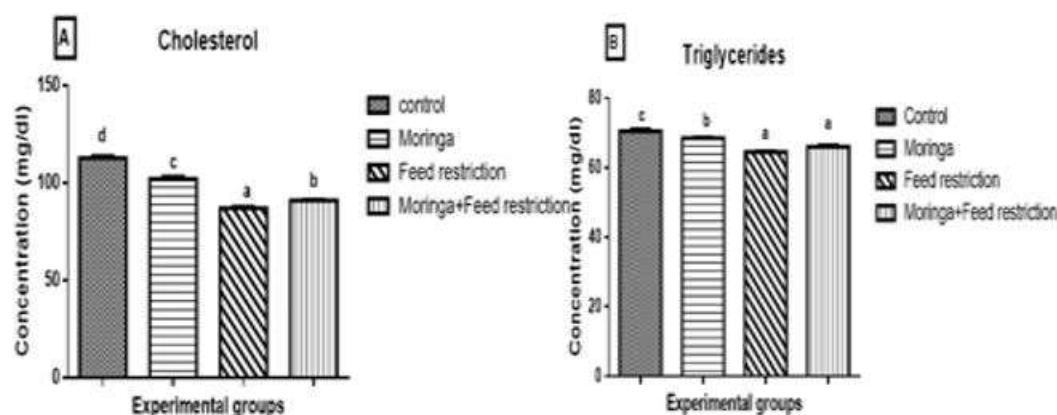
Throughout the experimental period, rats in the feed restricted group (70% standard diet without *Moringa oleifera*) exhibited a significant reduction in body weight compared to the control group receiving 100% of the standard diet ( $p < 0.05$ ). This confirms the expected effect of dietary restriction on growth. Conversely, rats receiving *Moringa oleifera* supplementation at 500 mg/kg BW, whether on a full or restricted diet, showed significantly higher body weights than the feed restricted group ( $p < 0.05$ ). The group fed a restricted diet with *Moringa* supplementation demonstrated partial recovery in body weight, with values significantly greater than the restricted-only group but still slightly lower than the control. The group receiving *Moringa* with a full diet showed body weights comparable to the control group, indicating no adverse or excessive gain due to supplementation. These differences are illustrated in **Fig. 1** with statistical significance denoted by different superscript letters ( $p < 0.05$ ).



**Fig. 1:** Effect of feed restriction either alone or with moringa supplementation on body weight in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ )

### Lipid profile

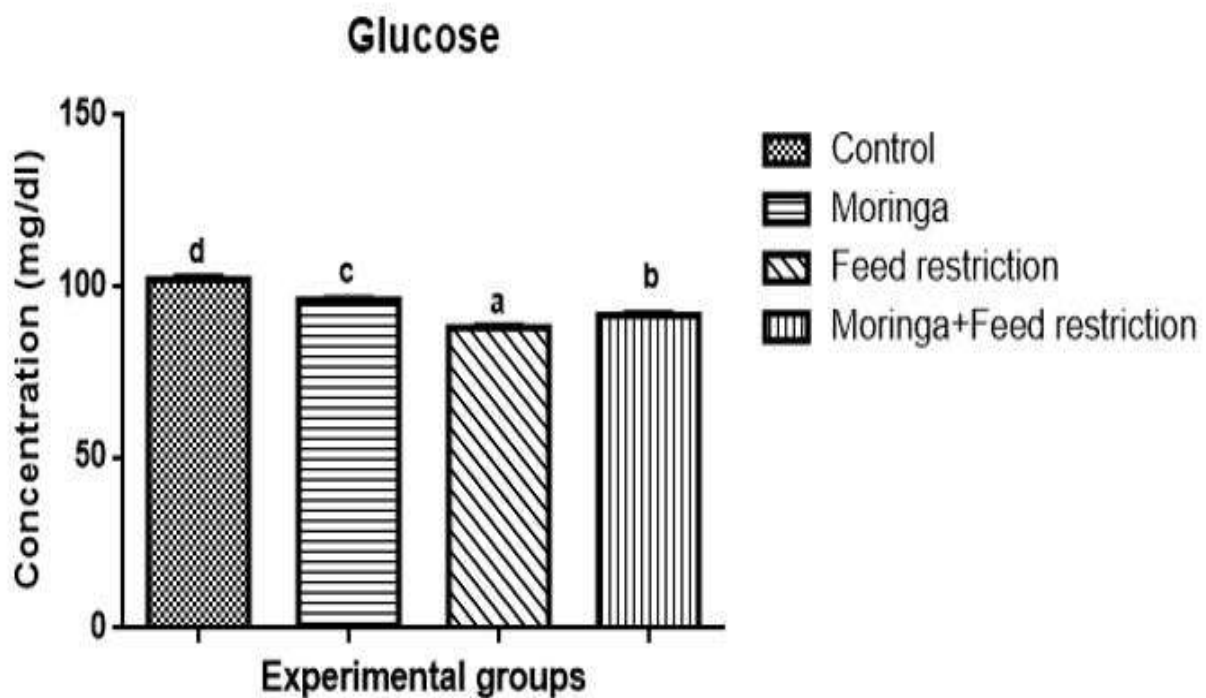
Rats in the feed restricted group (70% diet without *Moringa oleifera*) exhibited a significant reduction in serum total cholesterol and triglyceride levels compared to the control group ( $p < 0.05$ ), indicating the metabolic impact of caloric restriction. Supplementation with *Moringa oleifera* at 500 mg/kg BW in feed restricted rats led to a significant improvement in both cholesterol and triglyceride levels compared to the restricted-only group ( $p < 0.05$ ), though these values remained slightly lower than those of the control. Similarly, rats receiving Moringa supplementation with the full diet also showed modest reductions in lipid levels compared to control, but the differences were not statistically significant. Overall, *Moringa oleifera* attenuated the hypolipidemic effects of feed restriction, as shown in **Fig. 2** with statistically significant differences indicated by different superscript letters ( $p < 0.05$ ).



**Fig. 2:** Effect of feed restriction either alone or with moringa supplementation on (A) cholesterol, (B) triglycerides in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

### Plasma glucose level

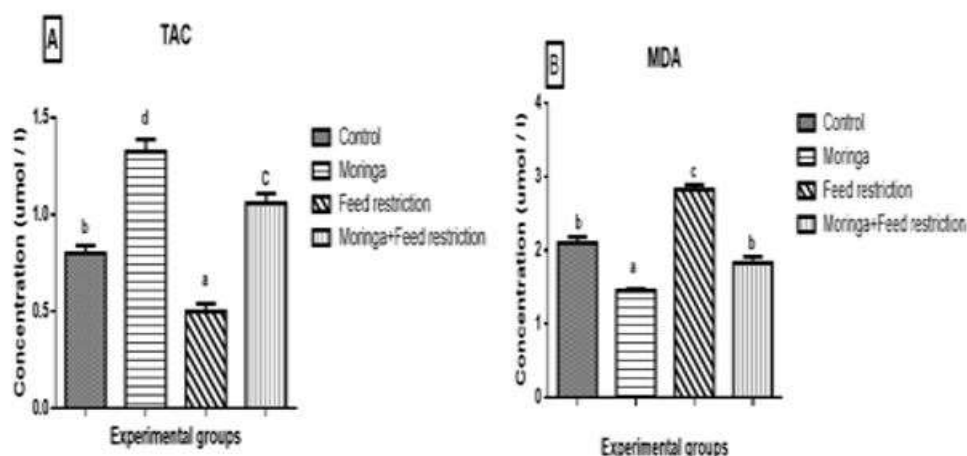
Fasting plasma glucose levels were significantly reduced in all treatment groups compared to the control group ( $p < 0.05$ ). The most pronounced decrease was observed in the feed restricted group, which had the lowest glucose levels among all groups ( $p < 0.05$  vs. control and other groups). Rats receiving *Moringa oleifera* supplementation alone (on a full diet) also showed a significant reduction in glucose levels compared to the control group, though the reduction was less marked than in the restricted group. Notably, the group receiving both feed restriction and *Moringa* supplementation demonstrated intermediate glucose values significantly higher than the restricted only group but still lower than the control group ( $p < 0.05$ ). These findings indicate that *Moringa oleifera* mitigates the hypoglycemic effect of dietary restriction. Statistical differences are illustrated in **Fig. 3** with distinct superscript letters indicating significance ( $p < 0.05$ ).



**Fig. 3:** Effect of feed restriction either alone or with moringa supplementation on plasma glucose level in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

### Oxidative stress and antioxidant status

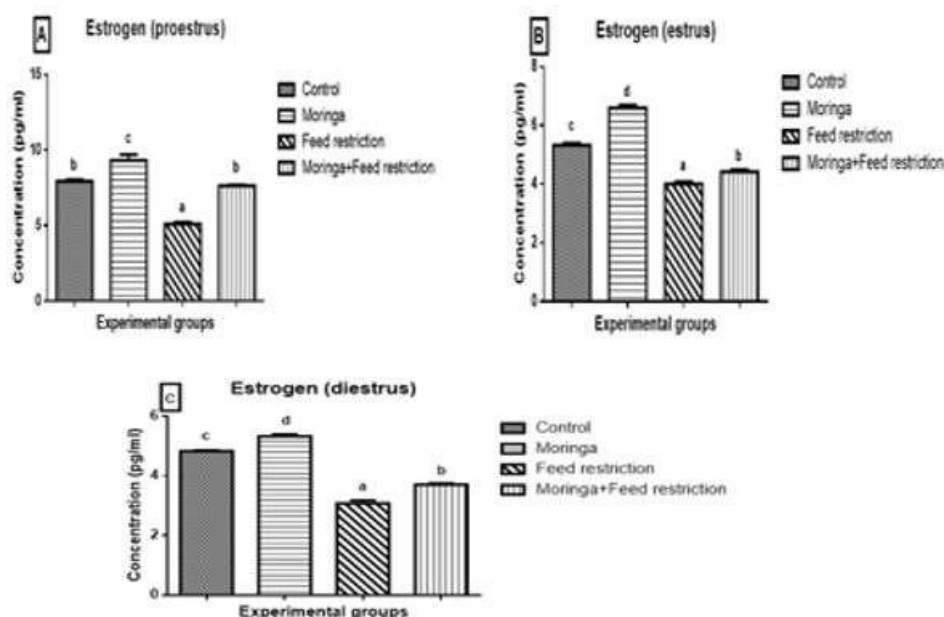
Feed restriction alone resulted in a significant decrease in total antioxidant capacity (TAC) and a marked increase in malondialdehyde (MDA) levels compared to the control group ( $p < 0.05$ ), indicating elevated oxidative stress. Supplementation with *Moringa oleifera* (500 mg/kg BW) significantly improved antioxidant status. Both the Moringa-supplemented full diet group and the feed restricted + Moringa group showed significantly higher TAC levels than the restricted only group ( $p < 0.05$ ). Although TAC in the restricted + Moringa group remained slightly lower than the control, the improvement was statistically significant compared to feed restriction alone. Similarly, MDA levels were significantly reduced in the Moringa supplemented groups compared to the feed restricted group ( $p < 0.05$ ), indicating reduced lipid peroxidation. MDA levels in the restricted + Moringa group approached those of the control group, suggesting a protective effect of *Moringa oleifera* against oxidative stress. These findings are presented in **Fig. 4** with different superscript letters denoting statistically significant differences ( $p < 0.05$ ).



**Fig. 4:** Effect of feed restriction either alone or with moringa supplementation on (A)TAC and (B) MDA in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

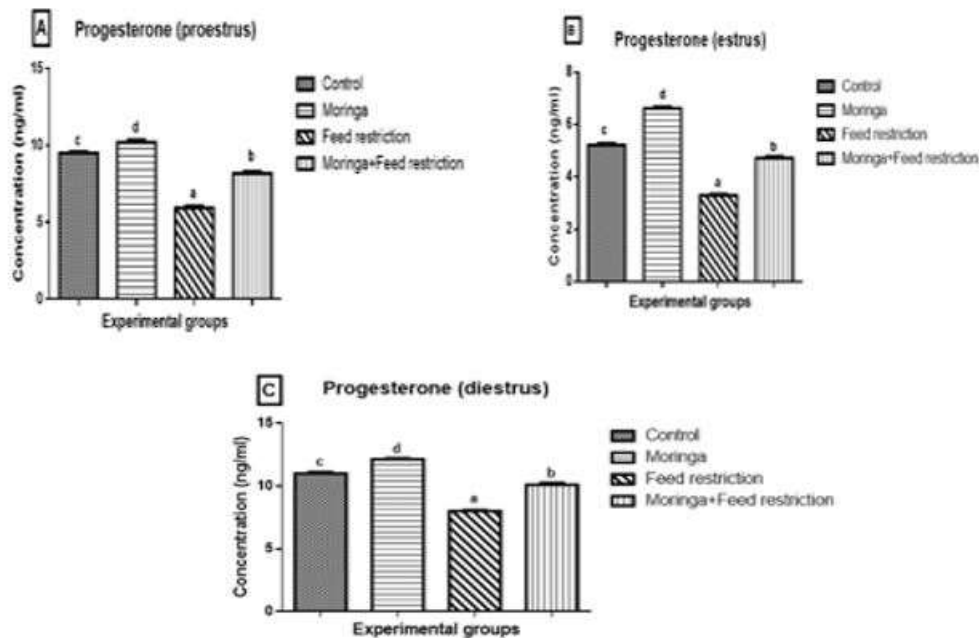
### Serum reproductive hormones, estrous cycle assessment via vaginal cytology

Vaginal cytology analysis revealed distinct alterations in the estrous cycle phases across the experimental groups. In the control group, regular cyclicity was maintained, with all animals displaying the characteristic sequential stages of proestrus, estrus, metestrus, and diestrus. In contrast, the feed restricted group exhibited irregular cycles and prolonged diestrus phases, with cytological smears predominantly composed of leukocytes and few cornified epithelial cells, indicating suppressed ovarian activity. Moringa supplementation in feed restricted rats partially restored estrous cyclicity, with vaginal smears showing increased frequency of cornified epithelial cells and intermediate cells suggestive of proestrus and estrus phases. Animals supplemented with Moringa under normal feeding conditions maintain regular cycles comparable to the control group.



**Fig. 5:** Effect of feed restriction either alone or with moringa supplementation on estradiol levels in different phases of estrous cycle in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

Serum levels of estradiol and progesterone were significantly reduced in the feed restricted group compared to the control group across all phases of the estrous cycle ( $p < 0.05$ ), indicating disruption of normal reproductive hormonal balance due to caloric restriction. Supplementation with *Moringa oleifera* (500 mg/kg BW), whether in normally fed or feed restricted rats, resulted in significantly higher levels of both hormones compared to the feed restricted group ( $p < 0.05$ ). In the group receiving Moringa with a full diet, hormone levels were comparable to the control group, with no significant differences detected. Meanwhile, in the restricted + Moringa group, estradiol and progesterone levels were partially restored, showing significant improvement over the restricted only group, though still slightly lower than the control ( $p < 0.05$ ). These results suggest that *Moringa oleifera* mitigates the negative impact of feed restriction on female reproductive hormones. Data are illustrated in **Fig 5 and 6** with statistically significant differences indicated by different superscript letters ( $p < 0.05$ ).



**Fig. 6:** Effect of feed restriction either alone or with moringa supplementation on progesterone levels in different phases of estrous cycle in female rats. Values are presented as mean  $\pm$  standard error (SEM). Bars marked with different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

## DISCUSSION

The present study demonstrates that dietary feed restriction in adult female rats induces significant physiological disturbances, including reductions in body weight, alterations in lipid and glucose metabolism, elevated oxidative stress, and disruption of reproductive hormone levels. These findings are in agreement with previous reports that highlight the detrimental effects of nutritional insufficiency on metabolic and reproductive homeostasis (**Bindari et al., 2013**).

The marked decline in body weight observed in feed restricted rats aligns with the results of **Zeng et al., (2019)**, who reported that caloric restriction reduces energy reserves and suppresses growth in female rodents. *Moringa oleifera* supplementation, however, significantly mitigated this weight loss. This beneficial effect may be attributed to its high content of protein,

essential amino acids such as lysine and methionine, vitamins, and trace elements that support tissue repair and growth (**Gopalakrishnan et al., 2016**).

Consistent with metabolic adaptations to negative energy balance, feed restriction in our model led to significant reductions in serum total cholesterol, triglycerides, and glucose levels. These effects have been attributed to suppressed hepatic lipid synthesis, including downregulation of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (**Redman and Ravussin, 2011**), enhanced lipid oxidation, and improved insulin sensitivity that reduces very low-density lipoprotein (VLDL) production and increases lipid clearance. Additionally, reduced hepatic gluconeogenesis and enhanced insulin action contribute to the decline in plasma glucose under restricted feeding conditions (**Lee and Longo, 2011**). *Moringa oleifera* supplementation partially reversed these alterations.

The hypolipidemic and hypoglycemic effects observed are likely mediated by its rich phytochemical profile, including flavonoids, saponins, and polyphenols, which modulate lipid metabolism and enhance insulin sensitivity (Mbikay, 2012). This is further supported by Helmy *et al.*, (2017), who reported lipid lowering effects of *Moringa* in hyperlipidemic rats. Moreover, the reduction in glucose levels observed in *Moringa* treated groups corroborates findings by Hong *et al.*, (2023) suggesting that *Moringa oleifera* enhances glucose transporter expression and insulin responsiveness mechanisms particularly beneficial in the context of feed restriction and its associated metabolic stress.

Oxidative stress is a well established consequence of undernutrition, and our results confirm this by showing decreased total antioxidant capacity (TAC) and increased malondialdehyde (MDA) levels in the feed restricted group. These findings are consistent with those of Walsh *et al.*, (2014), who reported increased oxidative damage under restricted dietary conditions. *Moringa oleifera*, known for its potent antioxidant properties due to high levels of vitamins C and E, flavonoids, and polyphenols, significantly restored TAC and reduced MDA in both normal and restricted diets. This antioxidant effect has also been demonstrated in recent work by El-Sherbiny *et al.*, (2024), reinforcing its tissue-protective capabilities. Also, Bakeer (2021) reported that natural feed additives increase antioxidative parameters and decrease animal stress.

Reproductive function was notably impaired by feed restriction, as indicated by the significant reductions in serum estradiol and progesterone levels during the estrous cycle. These hormonal changes are likely due to disruption of the hypothalamic-pituitary-gonadal axis, which is highly sensitive to energy deficits (Iwasa *et al.*, 2018). Remarkably, *Moringa* supplementation ameliorated these effects, likely through improved oxidative balance and nutrient status, both of which are critical for follicular development and luteal function. Additionally, the presence of phytoestrogenic compounds in *Moringa* may contribute to its hormone-modulating effects (Mutwedu *et al.*, 2022).

A major strength of this study is its novel model focusing on the consequences of feed restriction in adult females, an area that remains underexplored despite the recognized vulnerability of female reproductive physiology to energy deprivation. Unlike most existing literature that centers on male models or disease contexts, our work directly addresses real-world challenges such as malnutrition, dietary insufficiency, and production stress in females. Moreover, we demonstrate that *Moringa oleifera* at a low dose (500

mg/kg BW) offers a broad spectrum protective effect across metabolic, oxidative, and reproductive domains.

Our findings are consistent with previous studies that highlight the metabolic and antioxidant benefits of *Moringa* (Zeng *et al.*, 2019; Mbikay, 2012; Hong *et al.*, 2023), yet this study adds novel insights by linking these effects to the preservation of female reproductive hormones under caloric restriction. Additionally, we extend recent evidence of *Moringa*'s antioxidant potential (El-Sherbiny, 2024) by showing that these actions are effective in counteracting oxidative insults induced by dietary stress, as demonstrated by the improvements in TAC and MDA. While the current findings demonstrate the short-term protective effects of *Moringa oleifera* under feed restriction, it remains unclear whether these benefits are sustained over the long term or if continuous supplementation is necessary to maintain physiological balance. Therefore, extended-duration studies are needed to evaluate the persistence and stability of *Moringa*'s effects over time."

## CONCLUSION

The present study demonstrates that *Moringa oleifera* supplementation at a dose of 500 mg/kg body weight significantly mitigates the adverse effects of feed restriction in adult female rats. Feed restriction alone led to marked reductions in body weight, plasma glucose, lipid levels, antioxidant capacity, and reproductive hormone concentrations parameters critical for maintaining metabolic and reproductive homeostasis. *Moringa oleifera* effectively counteracted these disruptions by improving body weight, normalizing glycemic and lipid profiles, enhancing antioxidant defenses (as evidenced by increased TAC and reduced MDA), and restoring estradiol and progesterone levels during the estrous cycle. These findings underscore the potential of *Moringa oleifera* as a functional nutritional supplement to safeguard female reproductive and metabolic health during periods of caloric restriction, malnutrition, or stress-related feeding limitations. The model employed in this study offers valuable insights into sex-specific responses to nutritional challenges. Future studies are warranted to explore the molecular mechanisms underlying these effects, including gene expression and key signaling pathways, to further validate *Moringa*'s therapeutic promise in nutritional and reproductive science.

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