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Hormonal and Antioxidant Variations During and out Rutting Season in Camels

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Abstract | In the current study, the serum levels of enzymatic antioxidants including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) as well as hormonal factors such as cortisol, follicle-stimulating hormone (FSH), and estradiol, along with the non-hormonal factor malondialdehyde (MDA), were assessed in camels during and after the rutting season. Six she camels were used in this study in two different periods, the first out-rutting season in September and the second during the rutting season in January. Blood samples were collected from both periods and serum samples were tested for the main hormonal and antioxidant enzyme activity during and out of the rutting seasons. The findings demonstrated that while there was no discernible difference in CAT activity between the two periods, GSH-Px and SOD activity were much greater ($p < 0.05$) during rutting season than it was during the non-rutting season. MDA, a measure of oxidative stress, showed a non-significant variation in its level during and after the rutting season in the current research. The results of the hormonal analysis showed that there was no change in cortisol levels between the rutting and non-rutting seasons, but there was a considerable rise in FSH and estradiol levels. In conclusion, a high correlation was observed between the tested hormones and enzymatic antioxidant activity during the camel rutting season, which coincided with the onset of seasonality.

Keywords | Camels, Enzymatic antioxidant, MDA, Oxidative stress, Rutting season

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INTRODUCTION

Camels are widely distributed animals in the world due to their economic and therapeutic importance. This species is also considered to be of little interest to researchers compared to other animals, especially in the field of reproductive techniques and improving the breed, as well as artificial insemination and determining seasonality (Abrehaley and Leta, 2018). Like many other mammals, camels have an estrous cycle (reproductive cycle), which typically lasts around 20-22 days and may be extended to 28 days and can vary depending on many factors such as species, environmental conditions, and individual

physiology (Mohamed *et al.*, 2021).

Camel's estrous cycle differs from other animals because they are classified as induced ovulatory animals, and the act of copulation triggers the ovulation of this species (Mohamed *et al.*, 2021). The luteal phase is linked to ovulation; in the event that mating is unsuccessful, the estrus cycle is restricted to the follicular phase, and another cycle of estrus will start immediately without passing through the luteinizing phase (Skidmore *et al.*, 1996). Additionally, the estrous cycle of camels can vary between different species, such as dromedary camels (single-humped camels) and Bactrian camels (double-humped camels) (Alfurajji, 1999).

Depending on the species and climatic conditions, camel breeding seasons might differ. In camels, the mating season usually takes place in the fall or winter, when the weather is more temperate, and throughout the colder months of the year (December to April) (Ibrahim, 2008). One study's observations revealed that a well-fed female camel exhibit limited ovarian activity during the hot summer months. The seasonality in conception is determined by a decrease in male libido or sexual activity accompanied by higher rates of early embryonic death as the temperature rises (Skidmore, 2011).

The hormonal control of the estrous cycle in camels, like in other mammals, involves a complex interplay of various hormones that regulate the reproductive processes. However, camels exhibit some unique characteristics in their hormonal control compared to other mammals. Generally, camels are classified as an induced ovulatory animal and the act of copulation triggers the ovulation of this species during estrus phase (Mohamed *et al.*, 2021). The burst follicle develops into the corpus luteum, a tissue that secretes progesterone, following ovulation. Because it facilitates early gestation and primes the uterus for embryo implantation, progesterone is necessary to sustain pregnancy.

Camels' health and wellbeing are greatly aided by antioxidants, particularly in seasons when the environment is more stressful (El-Khasmi, 2024), and supporting the immune system during seasons (El-Sayed *et al.*, 2024), and contributes to the camel's ability to thrive in their natural habitat throughout the year (Abdel-Rahim, 2005). This mechanism occurred by neutralizing free radicals and supporting various physiological functions (El-Khasmi, 2024; El-Sayed *et al.*, 2024). Certain antioxidants, like vitamin E and the element selenium have been shown to improve animal reproductive health. These nutrients can help maintain the integrity of reproductive cells and support fertility. In seasonal breeding species like camels, having adequate levels of antioxidants in their diet during the breeding season can contribute to reproductive success (El-Sayed *et al.*, 2024; El-Hamid, 2021).

Antioxidants play an important role during the breeding season in sheep, goats, mares and buffaloes in terms of their essential role in improving hormonal effectiveness and reproductive function, as well as their essential role in controlling oxidative factors, which are relatively associated with harsh environmental conditions (Chainy *et al.*, 2016). The purpose of this study was to examine the relationship between antioxidants and oxidative stress with reproductive hormones during and outside the rutting season in camels in southern Iraq, as there have been few scientific studies on the subject.

This study primarily focused on the relationship between antioxidants and oxidative factors with seasonality in camels.

MATERIALS AND METHODS

STUDY PERIOD AND LOCATION

Six she-camels from Safwan desert, Basrah, Iraq, aged 5-10 years, were used for the current study from September /2023 to March/2024 in Basrah, Iraq. The camels were kept and housed under comparable food and management circumstances, with health monitoring by Veterinarians.

BLOOD SAMPLE

Using an 18 G needle and a sterile centrifuge tube, blood samples were taken from the animals' jugular veins twice. The first period (out of the rutting season) is during the dry, hot season (September). In contrast, the second period (in the rutting season) is during the wet winter (January), according to the study of (Al-Delemi, 2007). Serum was extracted from blood samples by centrifuging them for 20 minutes at 3000 rpm. After labeling, it is stored at -20 °C until the parameters of the research are analyzed.

ANTIOXIDANTS ANALYSIS

Glutathione peroxidase (GSH-Px) activity was measured at 412 nm and 37°C using a particular kit (Sza kits, Germany) in compliance with (Flohe and Gunzler, 1984). According to the (Aebi, 1984) methodology, Catalase enzyme (CAT) activity in serum (units/ml) was measured by using (Sza kits, Germany) at 240 nm after 30 seconds. The activity of superoxide dismutase (SOD) in serum was determined using a specialist kit (Sza kits, Germany) at 480 nm as soon as feasible at 37 °C based on (Flohe and Gunzler, 1984). Malondialdehyde (MDA) level (Mmole/L) was computed using the formula from (Beuge and Aust, 1978) and recorded at 25 °C and 532 nm. All analyses were done using a PD-303 UV spectrophotometer, APEL CO., Ltd, Japan.

HORMONAL ANALYSIS

The enzyme-linked immunosorbent assay (ELISA) method was used to measure the quantities of cortisol, FSH, and estradiol E2 using specific hormones commercially available kits (Chemux BioScience, Inc., San Francisco, USA) and according to the manufacturer's instructions (Alsalm *et al.*, 2023). For estradiol 17-β, the test's sensibility, intra-, and inter-assay precisions were 10 pg/mL, cortisol, 5.5 pg/mL, and FSH, 0.1 μIU/mL.

DATA ANALYSIS

Utilizing the independent samples t-test in the SPSS software program, antioxidant activity and hormone levels were compared between the non-rutting and rutting

seasons (Version 21, IBM, USA). The data were displayed as mean $\pm$ SEM, with statistical significance defined as p values < 0.05 .

RESULTS AND DISCUSSION

Antioxidant enzyme activity in camels was assessed, and the results indicated that these animals had significantly higher amounts of GSH-Px and SOD ($P < 0.05$) during the rutting season than during the non-rutting season. But between these two times, there was no change in the amounts of the catalase enzyme as shown in Figures 1 and 2. According to (Abdel-Rahim, 2005), the capacity of antioxidant enzymes to neutralize substances that produce reactive oxygen species (ROS) is a prerequisite for the antioxidative mechanisms of defense against oxidative stress. By causing oxidation or chemical change, ROS can interact with cells and organs, leading to a variety of illnesses for tissues and cells (El-Hamid, 2021).

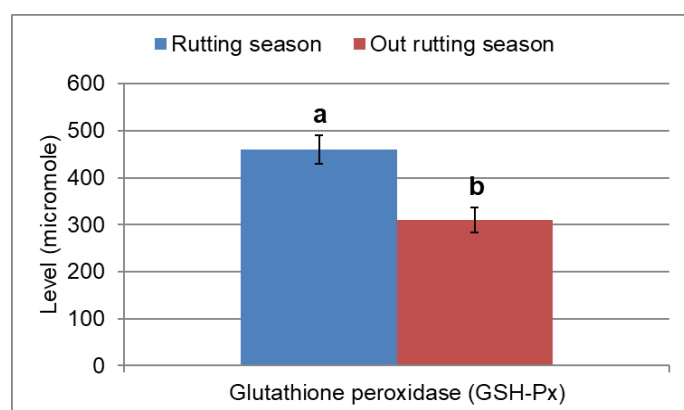


Figure 1: GSH-px activity ($\mu\pm$ SEM) in camels during and out rutting season. ^{a, b} Different letters indicate significant differences at $p < 0.05$.

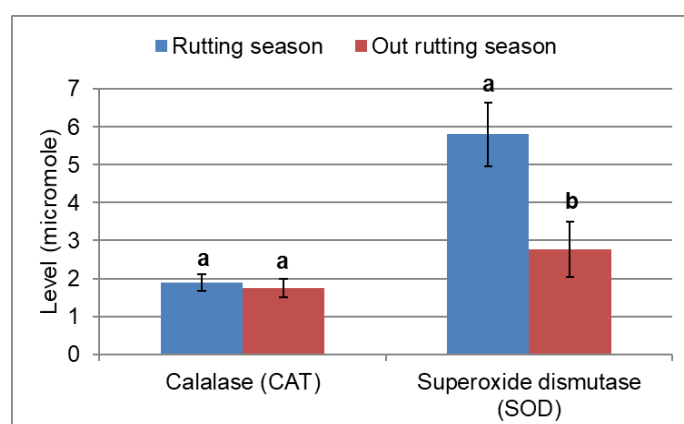


Figure 2: CAT and SOD activity ($\mu\pm$ SEM) in camels during and out rutting season. ^{a, b} Different letters indicate significant differences at $p < 0.05$.

During the rutting season, camels often exhibit a variety of physiological and hormonal changes, which are followed by the generation of free radicals. The body's demand

for antioxidants to reduce the negative effects of ROS is improved by the increase in antioxidant enzymes during the rutting season. Many studies showed the role of enzymatic antioxidants in many cases like heat stress (Bernabucci *et al.*, 2002), mastitis infection (Machado *et al.*, 2014), retained placenta (Kankofer, 2001), and cystic ovaries (El-Badry *et al.*, 2020) and its effect of minimizing the impact of ROS.

Additionally, the present investigation revealed no distinction in CAT activity ($p > 0.05$) between the rutting season and other times as in Figure 2. The non-significant difference in the CAT may be attributed to the role of other enzymes in controlling the free radicals production during this period without the need for the catalase enzyme (El-Badry *et al.*, 2020), or it may be attributed to the fact that the role of catalase may appear in the late stage of the rutting season, unlike the other enzymes (GSH_Px and SOD), whose effect seemed early in the rutting season (Bernabucci *et al.*, 2002).

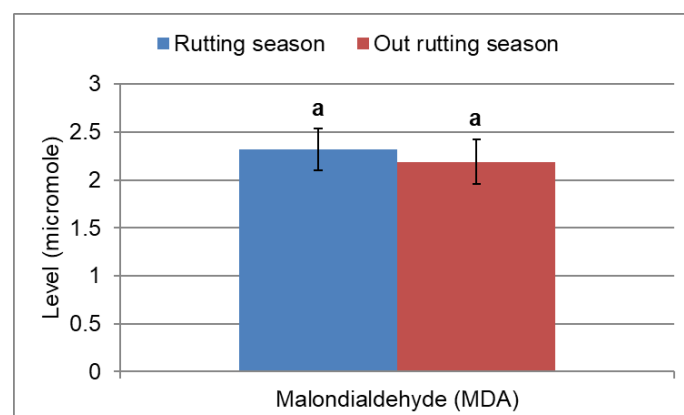


Figure 3: MDA level ($\mu\pm$ SEM) in camels during and out rutting season. ^{a, b} Different letters indicate significant differences at $p < 0.05$.

As seen in Figure 3, the indicator of oxidative stresses through MDA level was assessed in camels both during and after the rutting season in the research that is being done. The results showed a non-significant change ($p > 0.05$) in its level during and after the rutting season. In tissue systems, MDA serves as a measure of oxidative stress and an indication of lipid peroxidation. Many studies showed the elevation of MDA levels in the case of retained placenta (Khudhair *et al.*, 2021), post-partum anestrus, and inactive ovaries (Alsalim *et al.*, 2023). No change in the level of MDA during the rutting season confirms the action of enzymatic antioxidants, and their ability to reduce the effects of free radicals and prevent them from causing stress factors on animals (Bernabucci *et al.*, 2002). These results were also confirmed by our hormonal analysis of cortisol, which showed a non-significant difference in its concentration during and out rutting season, which indicates the camels were not under stress factor during

this period. Numerous researches on cows (Humphrey *et al.*, 1983) and camels (Ismail, 1987) demonstrated how cortisol, which rises in reaction to fighting stress and reducing its impact on the body, plays a part in stress.

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NOVELTY STATEMENT

This study presents the relationship between antioxidants and oxidative factors with seasonality in camels and their roles for releasing reproductive hormones which responsible for seasonality appearance in camels.

AUTHOR'S CONTRIBUTION

HAA: Perceived and designed the study and analyzed the data

HAA, MFA, NHS: Performed the experiments and wrote the manuscript

All authors read and approved the final manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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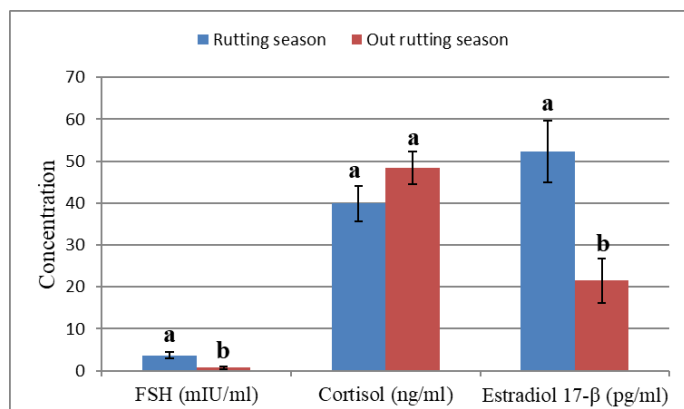


Figure 4: Concentration of hormones (FSH, cortisol, and estradiol) ($\mu\pm$ SEM) in camels during and out rutting season. ^{a, b} Different letters indicate significant differences at $p<0.05$.

When compared to non-rutting seasons, hormonal research showed a considerable rise ($p>0.05$) in FSH and estrogen during rutting season as shown in Figure 4. The seasonal reproductive activity in seasonal animals depends directly on the gonadotropin releasing hormone (GnRH) from the hypothalamus as well as FSH and LH hormones which are secreted from pituitary gland (Skidmore, 2011). The rutting season under many factors stimulates pituitary hormones to promote ovaries for folliculogenesis and production mature follicles which are responsible for production estrogen (Sathya *et al.*, 2007). Several studies have shown that camel reproductive activity starts from the December to March season, and that this is followed by a typical estrous cycle (Sathya *et al.*, 2007; Eiwishy, 1987). Reproductive hormones, particularly pituitary and hypothalamic hormones, significantly drop during the non-rutting season, and this is accompanied by a reduction in ovarian activity (Eiwishy, 1987). Our results supported earlier studies regarding how the season affects the cycle of reproduction in she-camel species.

CONCLUSION

The current study concluded that there is a close relationship between antioxidant levels and the entry to rutting season and the reproductive cycling. Furthermore, a harsh environmental condition during non-rutting season increases the oxidative stress along with declining in the reproductive hormones.

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