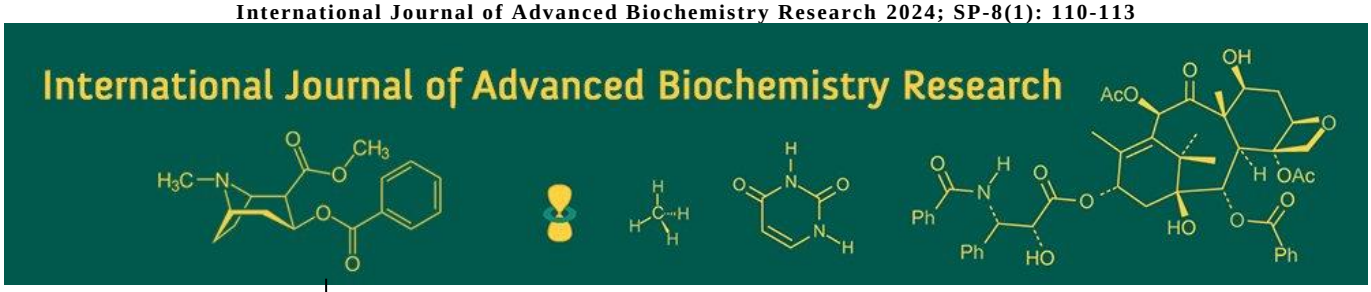

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Impact of dietary supplementation of *Moringa oleifera* leaves extract on oxidative indices of pubertal Marwari rams

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Abstract
The current investigation aimed to assess the serum total antioxidant capacity (TAC) of pubertal Marwari rams using an ethanolic dry extract of *Moringa oleifera* leaves (MOLEE). A total of twenty-four pubertal rams, aged 7-8 months and with an average body weight of 28 kg, were carefully chosen and randomly divided into four experimental groups, with six rams in each group. Group C received a standard diet without MOLEE supplementation, while groups T_1, T_2, and T_3 were provided with the same standard di*et al*ong with MOLEE at doses of 40, 80, and 160 mg/kg body weight respectively, for a duration of 90 days. Blood samples were collected every two weeks, and the serum was separated and stored at -20 °C for subsequent analysis of TAC using the ABTS decolorization assay. The findings revealed that the concentration of TAC (uM) was significantly (p<0.05) higher in groups T_3, T_2, and T_1 compared to group C. This study demonstrates that *Moringa oleifera* leaf extract can serve as a valuable feed supplement, enhancing sexual maturity and improving the overall antioxidant profile in pubertal Marwari rams.

Keywords: Pubertal, Ram, Moringa, sheep, antioxidant

Introduction
Small ruminants have a significant impact on the socio-economic and cultural well-being of rural communities in India. These animals provide livelihoods for two-thirds of the rural population, which constitutes 72.22% of the total population. The majority of these individuals are directly or indirectly dependent on agriculture and livestock-related occupations. Small ruminants are particularly suitable for small landholders and village systems due to their low initial investment, ease of rearing, and high feed conversion efficiency. Additionally, they exhibit remarkable adaptability to harsh climates, long migrations, resistance to tropical diseases, poor nutrition, and limited access to drinking water and water of good quality. Sheep, in particular, play a vital role in the Indian agrarian economy due to their multifaceted utility in providing meat, wool, skin, manure, and to some extent, milk. They are well-suited for arid and semi-arid tropics, thriving in marginal and sub-marginal lands (Bhateshwar *et al*., 2022) [3]. The early selection of males with high reproductive capacity plays a crucial role in efficient reproductive management and ultimately impacts the profitability of small ruminant flocks (Gouletsou and Fthenakis, 2010; Allaoui *et al*., 2014; Saaed and Zaid, 2018) [10, 2, 18]. The utilization of pubertal ram lambs can contribute to reducing production costs, enhancing the benefits of genetic selection, and facilitating earlier progeny and libido testing. Emphasizing the advancement of puberty in pubertal rams becomes a focal point in year-round breeding programs, enabling the exploitation of early fertility development and effective flock management (Khalifa *et al*., 2013) [12].

Moringa oleifera, a member of the Moringaceae family, possesses both medicinal and nutritional properties (Walaa *et al*., 2016) [23]. This plant is widely recognized in India, Pakistan, Bangladesh, and Afghanistan for its traditional medicinal uses (Mughal *et al*., 1999) [16]. The leaves of M. oleifera are abundant in various chemical components such as crude fiber, reducing sugars, resins, alkaloids, flavonoids, organic acids, sterols, tannins,

saponins, and proteins. Additionally, it has been found that *Moringa oleifera* is a rich source of antioxidants and polyphenols (Mishra *et al*., 2011) [14]. The phytochemicals present in *Moringa oleifera* include vanillin, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin. Furthermore, it is a valuable source of unsaturated fatty acids such as linoleic, oleic, and palmitic acids, as well as amino acids, vitamins, and minerals, particularly iron (Subadra *et al*., 1997; Faye *et al*., 2011) [21, 9]. For many decades, *Moringa oleifera* leaves have been used as a traditional medicine in numerous countries. It has also been recognized for its nutritional benefits, as it contains essential vitamins including A, B complex (B1, B3, B6, and B7), C, D, E, and K, among others (Shokry *et al*., 2020) [19]. The Moringa tree is a valuable source of easily digestible protein, calcium, iron, and antioxidants. Moreover, *Moringa oleifera* has been proven to possess hepatoprotective, antioxidant, anti-inflammatory, anti-tumor, diuretic, anti-pyretic, anti-ulcer, anti-hypertensive, anti-spasmodic, anti-diabetic, anti-microbial, and cholesterol-lowering properties (Abd El *et al*., 2014; Eldaim *et al*., 2017) [1, 6].

The current research has revealed that the extract from *Moringa oleifera* leaves has a positive impact on male reproductive function. It achieves this by reducing the presence of harmful free radicals in the sperm cytoplasm and enhancing the antioxidant capacity. This significant finding has prompted the current study to assess the effects of dietary supplementation with *Moringa oleifera* leaves on oxidative stress levels in pubertal rams.

Materials and Methods

The present study was carried out at ICAR-Central Sheep & Wool Research Institute (CSWRI), Arid Region Campus (ARC), Bikaner in collaboration with department of veterinary gynaecology and obstetrics, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner, Rajasthan, India.

Experimental animals and management

Twenty-four pubertal Marwari rams aged 7-8 months and with an average body weight of 28 kg were used in this study between July 2022 to September 2022. All rams were in good general health and clinically free of external and internal parasites. They were kept under standard conditions and fed a balanced ration that met the ICAR requirements for rams according to ICAR (2013) and were given water ad libitum around the clock.

Experimental design

Twenty-four pubertal rams (n=24) were randomly divided into four groups of six rams each. The first 10 days were considered the adaptation period. The rams in each group received a standard diet according to ICAR (2013), while the rams in the treatment groups received the standard diet together with an ethanolic extract of *Moringa oleifera* leaves for 90 days (July 2022-September 2022) after completion of the 10-day adaptation period.

Control group (C) was fed control/standard diet composed of Concentrate Feed Mixture (CFM) + Green/Dry Fodder only.

Second group (T_1) was fed control/standard diet and supplemented with *Moringa oleifera* leaves ethanolic dry extract @ 40 mg/kg B.Wt for 90 days.

Third group (T_2) was fed control/standard diet and supplemented with *Moringa oleifera* leaves ethanolic dry extract @ 80 mg/kg B.Wt for 90 days

Fourth group (T_3) was fed control/standard diet and supplemented with *Moringa oleifera* leaves ethanolic dry extract @ 160 mg/kg B.Wt for 90 days.

Collection of serum samples

Blood samples were taken fortnightly from the jugular vein. Samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes. The serum samples were separated and stored at -20 °C until a test was performed to determine the serum antioxidant profile.

Determination of serum antioxidant profile (Total antioxidant capacity) (TAC)

The total antioxidant capacity in serum samples were evaulated by ABTS (2, 2 V-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) decolorization assay and expressed in terms of Trolox equivalent (Erel, 2004).

Statistical Analysis

The statistical analysis of the obtained data was conducted using a 4×8 factorial design through analysis of variance. The computer program SPSS (version 20.0) was utilized, following the standard procedures outlined by Snedecor and Cochran (1994) [20]. To compare the mean values, the Duncan's Multiple Range Test (DMRT) as described by Duncan (1955) [5] was employed.

Results and Discussion

The overall mean value of TAC (uM) was recorded as 0.28±0.04, 0.31±0.06, 0.39±0.014 and 0.43±0.016 in groups C, T_1, T_2 and T_3 respectively (Table 1). In between groups TAC was recorded significantly (p<0.05) higher in MOLEE treated groups as compared to C group and recorded highest concentration in T_3 group. The TAC was also recorded at fortnightly interval and observed a significant (p<0.05) changes in MOLEE treated groups compared to C group with advancement of fortnight period and recorded maximum TAC concentration in T_3 and T_2 group in eight fortnight period (Table 1).

The findings of the current investigation align with the research conducted by Moyo (2012) [15], who observed that the oral administration of dried M. oleifera leaves (200 g/head/day) led to a significant increase in the antioxidant activity of GSH, SOD, and catalase, while also causing a notable decrease in lipid peroxidation. This study provided evidence suggesting that M. oleifera could serve as a valuable source of compounds with potent antioxidant properties. Similarly, the findings of Wafa *et al*. (2017) [22] support the present study, as they reported a significant enhancement in antioxidant enzyme activity such as catalase, glutathione (GSH), and superoxide dismutase (SOD) with dietary supplementation of Moringa leaves. Furthermore, the results of Shokry *et al*. (2020) [19] are consistent with the present findings, as they found that Moringa supplementation in barki rams resulted in significantly higher levels of seminal plasma catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase, alkaline phosphatase, acid phosphatase, ascorbic acid, and total antioxidant capacity. These outcomes are in line with the study conducted by Shorky *et al*. (2020) [19],

which demonstrated that *Moringa oleifera* leaves extract improved the quality of fresh and cryopreserved semen in Barki rams by enhancing the antioxidant defense system in seminal plasma. The findings of Oseni and Idowu (2014) [17] are in line with the results obtained in this study. They discovered that Moringa leaves extract enhanced the levels of antioxidant enzymes such as glutathione (GSH), glutathione peroxidase (GSH-Px), catalase, and total antioxidant capacity (TAC) in rats. Additionally, they found that the concentration of malondialdehyde (MDA), a marker of lipid peroxidation, was reduced. Similarly, Lamou *et al*. (2016) reported that the administration of aqueous Moringa extract at different doses (100, 200, or 400 mg/kg) improved the activity of antioxidant enzymes and decreased lipid peroxidation in rats. Another relevant study conducted by El-Gindy *et al*. (2017) [7] observed a significant enhancement in serum TAC and a decrease in MDA levels in rabbits that were fed diets containing Moringa extract, as compared to the control group.

The results of the study revealed that the antioxidant defense system mechanisms were positively influenced by the addition of moringa supplements. *Moringa oleifera* possesses potent antioxidant properties due to the abundance of vitamins C, E, and A, as well as caffeoylquinic acids, carotenoids, lutein, alpha-carotene, beta-carotene, kaempferol, quercetin, ellagic acid, and apigenin glucoside (Faye, 2011) [9]. These findings strongly indicate that the use of MOLEE shows great potential in enhancing semen quality.

Table 1: Effect of MOLEE on Total antioxidant capacity (uM) of Marwari Pubertal rams (Mean±SE)

Group Period	Control	T_1	T_2	T_3
I	0.22^{aA}±0.01	0.24^{aA}±0.01	0.23^{aA}±0.01	0.22^{aA}±0.01
II	0.26^{abA}±0.01	0.27^{aA}±0.01	0.28^{aAB}±0.01	0.30^{bB}±0.01
III	0.24^{abA}±0.03	0.28^{abA}±0.01	0.32^{bB}±0.01	0.36^{cC}±0.01
IV	0.28^{bcA}±0.01	0.30^{bcA}±0.01	0.39^{cB}±0.01	0.42^{dC}±0.02
V	0.29^{bcA}±0.01	0.32^{cB}±0.02	0.42^{dC}±0.01	0.46^{eD}±0.01
VI	0.25^{bcA}±0.02	0.34^{dB}±0.04	0.46^{eC}±0.01	0.50^{fD}±0.01
VII	0.31^{cA}±0.01	0.36^{dB}±0.02	0.50^{fC}±0.03	0.53^{gD}±0.01
VIII	0.30^{cA}±0.01	0.37^{eB}±0.01	0.55^{fC}±0.01	0.61^{hD}±0.01
Overall	0.28^{A}±0.004	0.31^{B}±0.006	0.39^{C}±0.014	0.43^{D}±0.016

Overall mean having different superscript in a row (capital letter A, B, C.) differ significantly (p<0.05)

Conclusion

Based on the results of the current investigation, it can be inferred that the addition of *Moringa oleifera* leaves ethanolic extract (MOLEE) to the diet of pubertal rams enhanced their overall antioxidant levels during the pubertal phase. Consequently, incorporating MOLEE into the diet of pubertal rams may facilitate early sexual maturation and enable their early utilization for breeding purposes.

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Conflict of Interest

None of the authors have any conflict of interest to declare.

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