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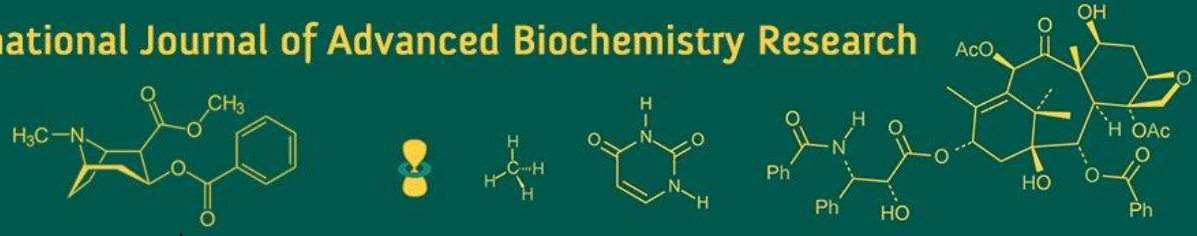

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Effect of moringa (*Moringa oleifera*) leaves extract administration on testosterone profile in Marwari pubertal rams

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Abstract
The current investigation aimed to examine the serum testosterone levels in pubertal Marwari rams were administered an ethanolic dry extract of *Moringa oleifera* leaves (MOLEE). A total of 24 pubertal rams, aged 7-8 months with an average body weight of 28 kg, were carefully chosen and divided into four experimental groups, each consisting of six rams. Group C received a standard diet without any MOLEE supplement, 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 testosterone analysis using vitros immunodiagnostic testosterone kits. The findings revealed that the testosterone concentration 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 beneficial feed supplement, enhancing sexual maturity and testosterone levels in pubertal Marwari rams.

Keywords: Pubertal, ram, moringa, sheep, testosterone

Introduction
Small ruminants hold significant importance in India rural population, contributing to their socio-economic and cultural well-being. These animals serve as a source of livelihood for two-thirds of the rural population. Sheep, in particular, are highly favorable for breeding due to their low initial investment and efficient feed conversion ratio. Additionally, they exhibit remarkable adaptability to harsh climates, possess an extended migration period, exhibit resistance to diseases, and offer a wide range of uses such as meat, wool, skin, manure and milk. These attributes contribute significantly to the Indian agricultural economy. Moreover, sheep are well-suited for arid and semi-arid regions with marginal and submarginal soils (Adegbeye *et al*., 2020) [2]. The reproductive performance of a flock is greatly influenced by the reproductive capacity of rams, which in turn affects its profitability (Shokry *et al*., 2020) [30]. It is crucial to select males with high reproductive capacity early on for effective reproductive management (Allaoui *et al*., 2014; Saaed and Zaid, 2018) [4, 29]. The success of rams in terms of reproduction depends on the quality and quantity of sperm as well as their libido (Perkins *et al*., 1992) [25]. By utilizing pubertal ram lambs, production costs can be reduced, genetic selection benefits can be promoted, and progeny and libido testing can be conducted at an earlier stage. Puberty in young male rams is defined as the age at which they exhibit sexual behaviors leading to mating and possess ejaculates containing live and viable sperm capable of fertilizing female ewes. Advancing puberty in pubescent rams offers advantages for year-round breeding programs, as it allows for early fertility development and efficient flock management (Khalifa *et al*., 2013) [15]. These strategies involve breeding animals at a young age to enhance their reproductive longevity, facilitate early progeny production, and expedite the testing of reproductive traits for accelerated genetic selection (Marai *et al*., 2009) [18]. The onset of puberty in small ruminants is influenced by a variety of factors, including the season of parturition, photoperiod, nutrition, management, genetics, hormonal regulation, body weight, and size (Mohamed *et al*, 2012; Ahsan *et al*, 2014) [21, 3]. The interaction between body weight, testicular growth, and testosterone secretion during

this stage is the most significant factor that affects puberty (Martinez *et al*, 2012) [19]. Nutrition, considered a crucial element among these factors, directly and indirectly regulates the attainment of puberty (Ahsan *et al*., 2014) [3]. Factors such as genotype, ewe experience, ram lamb/ewe ratio to body size, season, age, and feeding influence the development of testicles and the libido performance of rams or ram lambs (Mukasa-Mugerwa and Ezaz, 1992; Lindsay *et al*, 1993; Godfrey *et al*, 1998; Bielli *et al*, 2000; Simitzis *et al*, 2006) [23, 16, 11, 6, 31]. Male lambs that exhibit early puberty and display prominent sexual behavior have the potential to enhance the fertility of the flock during breeding, thereby leading to indirect genetic improvement (Ibarra *et al*., 2000) [12]. *Moringa oleifera*, a member of the Moringaceae family, possesses significant medicinal and nutritional properties as stated by (Walaa *et al*., 2016) [35]. It is renowned for its high levels of antioxidants and polyphenols (Mishra *et al*., 2011) [20]. The different components of M. oleifera, such as its leaves, roots, flowers, fruits, and seeds, contain a variety of beneficial phytochemicals including vanillin, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringin, kaempferol, and quercetin. Moreover, it is important to note the presence of unsaturated fatty acids like linoleic acid, oleic acid, and palmitic acid. Additionally, M. oleifera is rich in essential amino acids, vitamins, and minerals, particularly iron (Subadra *et al*., 1997; Faye, 2011) [32, 9]. Although MO has been shown to have positive effects on antirectile dysfunction and 6-ß-hydroxylation of testosterone (Monera *et al*., 2008; Prabsattroo *et al*., 2012) [22, 26], there is a lack of research on its influence on the reproductive performance of pubescent rams. Therefore, the objective of this study was to investigate the efficacy of an ethanolic extract obtained from MO leaves in increasing testosterone levels in pubertal Marwari rams.

Materials and Methods

The research was carried out at ICAR-Central Sheep & Wool Research Institute (CSWRI), Arid Region Campus (ARC), Bikaner and Department of Veterinary Gynaecology and Obstetrics at the College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), located in Bikaner, Rajasthan, India.

Experimental animals and management

Between July 2022 and September 2022, a total of twenty-four Marwari rams in their pubertal stage, aged 7-8 months and weighing an average of 28 kg, were selected for this study. These rams were in excellent overall health and showed no signs of external or internal parasites. They were housed under standard conditions and provided with a well-balanced diet that met the ICAR requirements for rams as specified by ICAR (2013) [13]. Additionally, they had unrestricted access to water at all times.

Experimental design

Twenty-four pubertal rams (n=24) were divided into four groups of six rams each in a random manner. The initial 10 days were designated as the adaptation period. During this time, all rams in each group were provided with a standard diet as per the guidelines set by ICAR (2013) [13]. Following the adaptation period, the rams in the treatment groups were supplemented with an ethanolic extract of *Moringa oleifera* leaves along with the standard diet for a duration of 90 days, specifically from July 2022 to September 2022.

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 hormone test was performed to determine the serum testosterone level.

Determination of serum testosterone level

Measurement of total serum testosterone levels of rams was performed with the Vitros Clinical Integrated System Analyzer (VT 5600 USA) using Vitros Immunodiagnostic testosterone kits according to the procedure prescribed by the manufacturer and measured in ng/dl.

Statistical Analysis

The statistical analysis of the obtained data was conducted using a 4×8 factorial design through analysis of variance. The computer programme SPSS (Version 20.0) was utilized, following the standard procedures outlined by Snedecor and Cochran (1994) [37]. To compare the mean values, the Duncan's Multiple Range Test (DMRT) as described by Duncan (1955) [36] was employed.

Results and Discussion

The present study was conducted to investigate the effect of oral supplementation with an ethanolic extract of *Moringa oleifera* leaves on serum testosterone levels in pubertal Marwari rams.

The mean value of total testosterone were reported as 1.40±0.04, 1.63±0.05, 2.76±0.14, and 3.46±0.22 in groups C, T_1, T_2, and T_3, respectively. When comparing the mean values between the groups, significant changes ($p<0.05$) were observed in the rams of T_3, T_2, and T_1 compared to C. The MOLEE supplementation group showed significant changes, with the highest changes observed in the T_3 group (Table 1). Total testosterone levels were also measured at fortnightly intervals, and a significant change ($p<0.05$) in total testosterone levels was observed between groups as the duration of the fortnightly period increased, with higher testosterone concentrations in the rams of T_3 and T_2 group (Table 1).

The findings of the present investigation are consistent with those of Dafaalla *et al*. (2016) [8], who found that the administration of *Moringa oleifera* leaf extract increased plasma testosterone levels in rats. Similar results were also reported by Syarifuddin *et al*. (2017) [33] and Ojo and Adetoyi (2017) [24], who observed a significant ($p<0.05$) rise in plasma testosterone levels in Bali bulls and male rabbits after supplementing with Moringa leaves. Iliyasu *et al*. (2020a) [14] further supported these findings by demonstrating that treating Yankasa rams with *Moringa oleifera* aqueous seed extract at a dosage of 1000-3000 mg/kg significantly ($p<0.05$) elevated testosterone, LH, and FSH profiles. The investigations conducted by Wafa *et al*.

(2017) [34], Prabsattroo *et al*. (2015) [27], and Dafaalla *et al*. (2016) [8] in rats align with these findings. Moringa, as discovered by Chauhan *et al*. (2014) [7], contains a variety of biocomponents that can enhance testosterone levels and promote spermatogenesis. These biocomponents consist of aphrodisiac properties, saponin, alkaloid, flavonoid, ferulic acid, and chlorogenic acid. Additionally, Ghasi *et al*. (1994) [10] found that Moringa contains β-sitosterol, which aids in preserving and improving spermatogenesis in mice. It is believed that plant extracts may contribute to the secretion of testosterone, thereby enhancing its availability to the gonads (Amini and Kamkar, 2005) [5]. Another potential mechanism of action for Moringa supplementation involves the increase in Leydig cells (Prabsattroo *et al*., 2015) [27] and the elevation of FSH and LH levels (Dafaalla *et al*., 2016) [8]. The synthesis of testosterone in the testes relies on the adequacy of dietary zinc (Roy *et al*., 2013) [28]. It is worth noting that *Moringa oleifera* leaves contain a sufficient amount of both zinc and β-sitosterol, which have been shown to preserve spermatogenesis in mice (Ghasi *et al*., 1994) [10]. However, a study conducted by Shokry *et al*. (2020) [30] found that the use of *Moringa oleifera* leaf extract led to increased semen volume and sperm concentration. Surprisingly, this improvement was not linked to testosterone levels, as there were no significant differences among the groups of Barki rams. The rise in testosterone levels indicates a stronger response of Leydig cells to gonadotropins, which are stimulated by the functioning LH receptor mechanism. This mechanism regulates the storage and release of testosterone. According to Mukasa-Mugerwa and Ezaz (1992) [23], factors such as body weight and age influence the concentration of testosterone in pubescent rams during puberty. Furthermore, Moringa supplementation has been reported to increase testosterone concentration, directly impacting secondary sexual activity. As a result, the increase in sexual activity, which is associated with elevated testosterone levels, has a direct effect on seminal characteristics (Mahmoud *et al*., 2013) [17]. The findings of this study suggest that *Moringa oleifera* leaf extract can raise testosterone levels in pubertal rams.

Table 1: Effect of MOLEE on Total testosterone (ng/dl) of Marwari Pubertal ram (Mean± SE)

Group Period	Control	T_1	T_2	T_3
I	1.07^{aA}±0.05	1.16^{aA}±0.03	1.35^{aA}±0.02	1.37^{aA}±0.03
II	1.12^{aA}±0.05	1.29^{abAB}±0.04	1.59^{aBC}±0.03	1.81^{bC}±0.05
III	1.20^{aA}±0.04	1.36^{abA}±0.04	2.24^{bB}±0.12	2.20^{cB}±0.17
IV	1.24^{aA}±0.04	1.50^{abcA}±0.07	2.53^{bB}±0.13	2.53^{dC}±0.29
V	1.37^{abA}±0.06	1.67^{bcdA}±0.1	2.95^{cB}±0.14	4.50^{eC}±0.27
VI	1.43^{abA}±0.07	1.85^{cdAB}±0.07	3.40^{dC}±0.16	5.29^{fD}±0.36
VII	1.75^{bcA}±0.08	1.98^{dA}±0.06	3.70^{dB}±0.11	5.88^{gC}±0.31
VIII	2.01^{cA}±0.11	2.26^{eA}±0.07	4.29^{eB}±0.21	6.81^{hC}±0.25
Overall	$1.40±0.04^{A}$	$1.63±0.05^{B}$	$2.76±0.14^{C}$	$3.46±0.22^{D}$

Mean having different superscript in a row (Capital letter A, B, C.) differ significanly (p<.05)

Mean having different superscript in a column (Small Letter a, b, c.) Differ Significanly (p<.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 resulted in an enhancement of their testosterone levels, leading to an increase in sexual activity at a young age. Consequently, incorporating MOLEE into the regular diet of pubertal rams can promote early sexual maturity, enabling them to be utilized for breeding purposes at an earlier stage.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors.

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