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Effect of dietary supplementation of *Moringa oleifera* leaves ethanolic extract on serum cholesterol level of pubertal rams

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Abstract

The primary objective of the present investigation was to evaluate the total cholesterol level of pubertal Marwari rams by utilizing an ethanolic dry extract derived from *Moringa oleifera* leaves (MOLEE). A total of twenty-four pubertal rams, aged between 7-8 months and with an average body weight of 28 kg, were meticulously selected and randomly divided into four experimental groups, each consisting of six rams. Group C received a standard diet without any MOLEE supplementation, while groups T₁, T₂, and T₃ were administered the same standard diet along with MOLEE at doses of 40, 80, and 160 mg/kg body weight respectively, for a duration of 90 days. Blood samples were collected biweekly, and the serum was isolated and stored at -20 °C for subsequent analysis of cholesterol using Erba test kits. The results indicated that the concentration of cholesterol (mg/dl) was significantly ($p < 0.05$) lower in *Moringa* supplemented groups in comparison to control. This study provides evidence that the extract of *Moringa oleifera* leaves can serve as a valuable dietary supplement, promoting sexual maturity and reducing cholesterol levels in pubertal Marwari rams.

Keywords: Pubertal, ram, *Moringa*, sheep, cholesterol

Introduction

Small ruminants play a significant role in the livelihoods of many small and marginal farmers as well as landless laborers. They make a substantial contribution to the agrarian economy, especially in regions where crop and dairy farming are not financially viable. In India, the total sheep population is 74.26 million, which represents a 14.13% increase from the previous livestock census (65.07 million). Rajasthan state ranks fourth in sheep rearing, with a population of 7.9 million (BAHS, 2020) [4]. Sheep rearing holds great importance in the rural economy, particularly in arid, semi-arid, and mountainous areas of the country due to its diverse utility for wool, meat, milk, skins, and manure. *Moringa oleifera*, also known as drumstick, horseradish, or benzoil tree, is a perennial tropical deciduous tree that is native to northwestern India and northeastern Africa. It is often referred to as the "Nature nutrition of the tropics" due to its rich nutritional content (Naidoo & Coopoosamy, 2011) [10]. The leaves of the *Moringa* plant are particularly valuable, as they are packed with protein, amino acids, vitamins, and minerals that fulfill the nutritional requirements of animals (Teixeira, 2014) [16]. In fact, dried *Moringa* leaves have been found to contain 30.3% crude protein and 19 different amino acids (Moyo *et al.*, 2011) [9]. Additionally, *Moringa* leaves are abundant in bioactive compounds, especially antioxidants such as phenols, flavonoids, proanthocyanidins, flavonols, vitamin C, vitamin E, β -carotene, zinc, and selenium. These antioxidants have been shown to possess strong antioxidant properties and play a crucial role in inhibiting and scavenging free radicals (Ahmad, 2006; Iqbal & Bhangar, 2006; Lako *et al.*, 2007) [1, 6, 7]. Furthermore, *M. oleifera* has been found to have various protective effects, including reducing liver lipid peroxides and acting as antimicrobial, antitumor, anti-inflammatory, antiulcer, antihyperlipidemic, antidiabetic, anticancer, and cholesterol-lowering agents (Paul *et al.*, 2018) [12]. Given the increasing interest in utilizing natural plants to enhance fertility and reproductive functions in animals, particularly for successful artificial insemination in sheep, this study aimed to investigate the beneficial effects of different levels of ethanolic extract of *M. oleifera* leaves (MOLEE) on serum cholesterol levels.

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 (T₁) was fed control/standard diet and supplemented with *Moringa oleifera* leaves ethanolic dry extract @ 40 mg/kg B.Wt for 90 days.

Third group (T₂) was fed control/standard diet and supplemented with *Moringa oleifera* leaves ethanolic dry extract @ 80 mg/kg B.Wt for 90 days

Fourth group (T₃) 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 and analysis

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 and determined by (EM 200 Germany) automated chemistry analyser by using Erba test kits, as per the manufacturer's described procedure.

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) [15].

Results and Discussion

The overall mean value of total cholesterol (mg/dl) was recorded as 64.35±1.16, 63.20±0.72, 61.60±0.66, and 59.89±0.63 in group C, T₁, T₂ and T₃, respectively (Table 1). In between groups total cholesterol (mg/dl) concentration was differed significantly ($p<0.05$) lower level in MOLEE treated groups as compared to C group and recorded lowest level in T₃ group. Total cholesterol concentration (mg/dl) was also recorded at fortnightly interval and recorded significant ($p<0.05$) decrease in concentration of total cholesterol (mg/dl) with advancement of fortnight periods in T₃ and T₂ group. (Table 1).

The study findings align with the research conducted by Babiker *et al.* (2021) [19], which observed that ewes and goats fed on diets supplemented with *Moringa oleifera* leaves for a duration of 6 weeks experienced a significant decrease in serum cholesterol levels ($p<0.05$). This reduction in cholesterol levels can be attributed to the high phenolic content and antioxidant properties of *Moringa oleifera* leaves. Phytochemicals and antioxidants have been shown to inhibit the synthesis and absorption of cholesterol (Saxena *et al.*, 2013) [14]. Similar results were found by Yadav (2022) [18], who discovered that the inclusion of *Moringa* in the diet significantly reduced cholesterol concentration in Marwari rams ($p<0.05$). Wafa *et al.* (2017) [17] also observed a significant decrease in cholesterol concentration in bulls supplemented with *Moringa oleifera* leaves. Additionally, they noted that the concentration of serum total cholesterol exhibited a fluctuating trend at different collection periods during the study. Astuti *et al.* (2011) [2] demonstrated that the consumption of *Moringa oleifera*, containing a certain amount of saponin, can have a positive impact on health. This is evident through the reduction of serum cholesterol levels and the maintenance of normal essential fatty acid concentrations. Similar findings were reported in studies conducted on rats by Pratik *et al.* (2013) [13], Lewis and Rader (2005) [8], and Nikkon *et al.* (2003) [11]. These studies revealed that *Moringa oleifera* leaves contain components that can regulate mechanisms involved in the elimination of lipids from the body. Furthermore, Lewis and Rader (2005) [8] observed a significant decrease in lipid profiles in rats fed *Moringa oleifera* leaves. Ghasi *et al.* (2000) [5] also noted that *Moringa oleifera* leaves are rich in the bioactive phytoconstituent β -sitosterol, which has cholesterol-reducing properties.

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

Group Period	Control	T ₁	T ₂	T ₃
I	63.66 ^{aA} ±1.12	66.00 ^{cAB} ±1.41	68.66 ^{eBC} ±1.15	69.33 ^{fC} ±1.02
II	63.00 ^{aA} ±0.73	68.66 ^{dC} ±1.45	65.83 ^{fB} ±1.49	68.83 ^{eC} ±0.98
III	63.16 ^{aA} ±1.17	66.50 ^{bB} ±1.8	64.66 ^{efAB} ±0.61	67.50 ^{eB} ±0.62
IV	62.33 ^{aA} ±1.05	64.33 ^{bcA} ±1.23	62.16 ^{deA} ±0.6	62.50 ^{dA} ±0.96
V	63.66 ^{aB} ±1.12	62.83 ^{bB} ±0.95	61.00 ^{cdB} ±0.82	57.66 ^{cA} ±1.05
VI	63.00 ^{aC} ±0.73	60.00 ^{aB} ±0.97	58.83 ^{bcB} ±0.75	52.66 ^{bA} ±0.88
VII	66.66 ^{bC} ±0.49	59.50 ^{aB} ±1.06	57.66 ^{bb} ±0.76	51.83 ^{bA} ±0.48
VIII	69.33 ^{bd} ±0.88	57.83 ^{aC} ±0.87	54.00 ^{aB} ±1.13	48.83 ^{aA} ±0.6
Overall	64.35 ^D ±1.16	63.20 ^C ±0.72	61.60 ^B ±0.66	59.89 ^A ±0.44

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

Mean having different superscript in a row (capital letter A, B, C.) and column (small letter a, b, c.) differ significantly ($p<0.05$) in fortnight period

Conclusion

According to the findings of the ongoing inquiry, it can be deduced that the inclusion of *Moringa oleifera* leaves ethanolic extract (MOLEE) in the diet of adolescent rams led to a decrease in cholesterol levels during their adolescence. As a result, the incorporation of MOLEE into the diet of adolescent rams might aid in advancing sexual maturation and enhancing the quality of their semen.

Conflict of Interest

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

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