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The biological role of clove oil in the diet of mature male rabbits on the physiological body functions, oxidative stress and physical semen properties

A. A. M. Habeeb*, M. A. A. Atta, A. I. A. Elhanafy, and A. K. Sharaf
Biological Applications Department, Nuclear Research Center,
Egyptian Atomic Energy Authority
Corresponding e-mail : alnaimy252011@gmail.com

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ABSTRACT

In an eight-week experiment conducted throughout the months of January and February, 2023, the goal of this study was to determine how changing the diet of mature male rabbits to include clove oil affected their body's physiological processes and the quality of their sperm. In this study, 24 mature rabbit bucks that were 8 months old and weighed an average of 2.650 g in the first production year were used. In a final randomized plan, the bucks were randomly assigned to two experimental groups, each with twelve rabbits. Bucks served as the control group in the first group and were fed a commercial pelleted food without any supplements. Each buck in the second group received one milliliter of clove along with the daily diet requirement from commercial pellets. Every two weeks, values for weight gain, feed consumption, testosterone levels, blood constituents, libido, and semen quality were assessed. The findings demonstrated that including clove oil in the diet of bucks rabbits resulted in a significant ($P<0.01$) improvement in each of daily feed intake, daily weight gain, testosterone hormone, and γ-globulin and a significantly ($P<0.01$) reduced glucose, cholesterol fraction levels in comparison to the control group. Additionally, adding clove oil increased ($P<0.01$) the rabbit bucks' physical semen attributes values. It was determined that including clove oil in the diet of male rabbits improved physiological body processes, produced hypercholesterolemia, and raised the quality of the rabbits' sperm without having a negative impact on liver or kidney function.

Keywords: Cholesterol, Clove oil, Rabbits, Semen quality, Testosterone.

INTRODUCTION

The majority of nations experience a beef shortage. Rabbits have been crucial in addressing the lack of meat production. This is due to the rabbits' quick growth and reproduction rates, early maturing, improved feed consumption, short generation times, strong genetic selection potential, variety of food alternatives, confined essential area, and simplicity of raising, especially in developing nations (El Saidy *et al.*, 2016; Habeeb *et al.*, 2019). Because there is not

enough feed available for the production of rabbits, efforts have been made to supplement animal feed with feed spices in order to boost the animals' nutritional value, growth rate, and feed efficiency while also reducing feeding expenses (El-Nomeary *et al.* 2015 and 2016).

Reactive oxygen species (ROS) are different types of activated oxygen, including free radicals like superoxide ions and hydroxyl radicals, as well as non-free radicals like hydrogen peroxide (Aqil *et al.*, 2006). Antioxidants are thought to quench free radicals and include endogenous enzymes such as glutathione peroxidase (GPX) and superoxide dismutase (SOD). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potential as sources of natural antioxidants, like the components of clove oil, which are well known to have potential antioxidants (Mu'nisa *et al.*, 2015). Free radicals and reactive oxygen species produced by every oxidative metabolism have the potential to kill an organism's cells in animal bodies (Krapfenbauer *et al.*, 2003). Antioxidants inhibit oxidation and scavenge free radicals and ROS; they are complexes that are used to lower the production of free radicals in the body. Exogenous antioxidants, especially natural antioxidants, must be added to the animal body to prevent excessive ROS production and fight free radicals during oxidative metabolism (Vercruysse *et al.*, 2009). Antioxidants can help farm animals be healthier and more productive by counteracting the damaging effects of oxidative stress by lowering free radicals, or ROS, in rabbits (Al-Shanty, 2003; El-Desoky *et al.*, 2017).

Essential oils, which are complex combinations of aromatic chemicals collected by distillation from diverse parts of plants, have garnered particular attention in recent years as biologically active plant extracts (El-Kholy *et al.*, 2022). Numerous studies have concentrated on the naturally occurring antioxidants in popular, widely used, and patronized therapeutic plants (Souza *et al.*, 2019). There is evidence from multiple studies that the clove (Syzygium aromaticum), a fragrant dried flower bud of the tree, has several elements, namely the eugenol compound (78%), beta-caryophyllene (13%), and fixed oil (10%) (Iqbal *et al.*, 2022; Chaieb *et al.*, 2022). Because cloves are so nutritious, their essential oil is used to boost health (Iqbal *et al.*, 2022). The oil is a clear to pale yellowish fluid that is heavier than water and soluble in alcohol. It is produced by steam distilling clove buds (Milind and Khanna, 2011). One plant extract, clove and its essential oil, has been shown to promote growth performance, suppress some intestinal infections, function as an antiseptic, stimulate digestion, and have antioxidant benefits (Nehme *et al.*, 2021; Bisergaeva *et al.*, 2021). Essential oils have been shown to improve gut health and digestion, supporting their usage in animal diets as natural growth boosters (Al-Mufarrej *et al.*, 2019). Additionally, the antioxidant compounds in aromatic plants may be the primary cause of their medicinal effects (Giannenas *et al.*, 2013). Due to its high and diverse content of approximately 36 bioactive constituents and phenolic compounds, such as eugenol, eugenol acetate, β-caryophyllene, caryophyllene oxide, and α-humulene, clove oil has garnered a lot of attention (Issac *et al.* 2015; Cortés-Rojas *et al.* (2014); Jimoh *et al.* 2017 (Chaieb *et al.*, 2007; Al-Shaikh and Perveen, 2017; Batiha *et al.* 2020; Iqbal *et al.*, 2022). Examining how clove oil impacts mature rabbits' physiological bodily functions and the physical quality of their semen in Egypt is the goal of the study.

MATERIALS AND METHODS

Experimental Site and Ethics

The practice of this research was supported at the Rabbits Farm, which is a part of the Experimental Farms Project, Atomic Energy Authority, at Inshas, Egypt (latitude 31°12′ N to 22°2′ N, longitude 25°53′ E to 35°53′ E), during the months of January and February of the winter season of 2023.

The Egyptian Atomic Energy Authority's animal care and welfare committee approved the study. Ethical clarification for animal research post conduction No 61A/22 at 26-12-2022. This

section discusses relevant data regarding efforts to lessen animal suffering and adherence to the strictest veterinary precautions.

Rabbits Feeding and Management

During the course of the trial, the buck rabbits of the two experimental groups received the same diet. Clover hay (40%), wheat bran (25%), yellow maize (15%), soybeans (10%), molasses (5%), bone meal (2%), calcium carbonate (1%), salt chloride (1%), vitamins and minerals premix (0.5%), and DL-methionine (0.5%) are the ingredients of the marketable food. The marketable food's chemical analysis shows that the DM percentages of crude protein, crude fiber, ether extract, nitrogen-free extract, and ash are respectively 18.5, 12.5, 3.5, 56.0, and 9.5%. A further 2600 kcal/kg DM of digestible energy is present.

Bucks Housing

Each buck was housed in a galvanized metal hutch measuring 60 by 60 by 60 cm and rising 100 cm above the ground. Before the bucks were transferred, the hutches were scrubbed, cleaned, and left for a week to dry. A nipple for drinking and a feeder for feeding the marketable food were also provided for each cage. All experimental groups received free access to food and water. Feces and urine are released from pens and cleaned every day. Clostridium enterotoxaemia bloat was used to protect the rabbits before the experiment.

Experimental Design

Twenty-four mature New Zealand White bucks, 6 months old and had an average live body weight of 2.650 g in the first production year were employed in this research. After randomization, the bucks were carelessly divided into two groups with twelve rabbits each. Bucks served as the control group in the first group and were fed a commercial pelleted food without any supplements. Each buck in the second group received the recommended daily amount of commercial pelleted feed together with one milliliter of love oil. For two months, the chosen dose of clove oil was provided to individuals who needed it as part of a commercial diet. Each two weeks, individual buck was weighed and feed intake was estimated. Extra virgin clove oil (AGEBA Press Production) that has been cold-pressed and is 100% pure is purchased in a 500-ml bottle from a public supermarket in Zagazig, Egypt.

Blood Biochemical Components and Testosterone Level Determination

During the two-month trial period, one blood sample was taken from each buck every two weeks. Each animal had five ml of blood drawn from the vein in its ear into a fresh, sterile tube without the use of anticoagulants. Blood samples were allowed to coagulate before being centrifuged at 3.000 rounds per minute for 25 minutes to obtain the serum (the supernatant). Up until the time of the analysis of the blood's biochemical components and testosterone concentrations, serum was kept frozen at a temperature of –20°C. Every two weeks, the serum was examined using commercial Chemical Reagent Kits for blood glucose, γ-globulin, total cholesterol, HDL and LDL concentrations, as well as liver function (ALT & AST enzyme activity) and renal function (urea and creatinine levels). Serum antioxidant glutathione peroxidase (GPx), and superoxide dismutase (SOD) were determined by using commercial kits (Bio-Diagnostic, Egypt) according to the procedure outlined. Every two weeks, radioimmunoassay (RIA) was used to measure the hormonal level of testosterone using a commercially available kit (Diagnostic Product Corporation, Los Angeles, USA). Iodine-125 was used to mark the testosterone hormone's tracer (I125). The fluid components in the tubes are removed after the incubation period, and the radioactivity of the labeled iodine is measured by a computerized gamma counter at the Egyptian Atomic Energy Authority's Biological Applications Department.

Semen Collection and Estimation of Semen Quality

For one week, the experimental bucks were allowed to get used to the semen collection be-

fore being trained to release and collect semen through an artificial vagina while attempting to mount four mature females as a teaser handling one week prior to the semen collection. During the experimental trial, a single semen sample was taken from each buck as the first ejaculate every two weeks by an artificial vagina employing a female teaser rabbit. A white Vaseline lubricant was used to lubricate the inner rubber sleeve of the prosthetic vagina, which had its temperature set at 40°C. At 10:00 a.m., semen was collected, and the gel was removed right away and quantified. To determine the quality of the semen, all samples were stored in a water bath (35–37 °C) and taken right away to the lab as follows:

After removing the gel mass, the amount of ejaculate (in mL) was measured using a graduated collection tube attached to the end of the artificial vagina. Using a light microscope with a heated stage and visual examination at 100X magnification, the percentage of sperm motility was calculated right after semen collection in numerous microscopic fields and ranged from 0 to 100%. Following semen dilution (1:100), the improved Neubauer hemocytometer slide (Hamburg, Germany) was used to measure sperm concentration (106/ml). Scores for semen mass motility ranged from 0 to 3. To determine the sperm viability percentage, a dried smear of a drop of each semen sample was stained with an eosin-nigrosin blue staining solution (live or dead). According to the staining outline, the sperm cells were divided into stained (dead sperm) and non-stained (live sperm) categories. The percentage of sperm abnormalities was assessed while counting 200 sperm cells and examining the live/dead sperm percentage under a high power (400X) microscope. The reaction time (measured in seconds) between when the buck was given the teaser (a female rabbit) and when the buck jumped and ejaculated the first copulation was also measured to determine libido (sexual desire or reaction time).

Statistical Analysis

The general linear model procedure of SAS (2002) was used to expose data to the analysis of variance (2002). The following is the statistical process: $Yij = \mu + Ti + Sj + eijk$ where Yij= the observation, μ = overall mean, Ti = the fixed effects of treatment (1=control, 2=clove oil), Sj= the fixed effects of sampling time (2, 4, 6 and 8 weeks) and eijk = random error. P values of 0.05 or lower were recognized as significant differences. The substantial discrepancies between means were examined using Duncan's Multiple Range Test (Duncan, 1955). The Chi-square test was used to examine the potential of semen viability and motility percentages, and significant results were assessed using numerous Z-tests to parallel equal amounts. Results were presented as means and standard error of the mean.

RESULTS AND DISCUSSION

Effect of Clove Oil on Live Body Weight and Feed Intake of Mature Male Rabbits

Table 1 provides information on the effects of clove oil on average body weight, total gain over 56 days, and average daily gain. After two, four, six, and eight weeks, respectively, the live body weight increased by 90, 130, 300, and 350 g as a result of the clove oil treatment. Additionally, the treatment with clove oil increased the total weight growth (g/56 days) and the average daily gain (g/day) by 250 g/56 days and 6.25 g/day, respectively.

The information in Table 2 explains how clove oil affects daily feed intake, total feed intake, and g/56 days. Following treatment with clove oil, the daily feed intake during the first, second, third, and fourth two weeks was increased by 10, 20, 20, and 25 g, respectively. Additionally, by treating with clove oil, both the average feed intake and the total feed intake (56 days) of each buck are improved significantly by 906 and 19 g, respectively.

Bucks who consumed clove oil in their diet ate more and put on more weight. Due to the use of clove oil, live body weights increased. The use of clove oil also increased both the overall weight gain and the average daily gain. Additionally, by using clove oil to treat, the total and

Table 1. Effect of adding clove oil to the diet of mature male rabbits on bucks' body weight

Body weight	Control	Clove oil	Increase in live body weight, g	Daily gain increase, g
No of bucks	12	12	-	-
Initial live body weight, g	2650±13	2650±15	-	-
Body weight after 2 weeks, g	2710^{b}±25	2800^{a}±27	90	6.43*
Body weight after 4 weeks, g	2830^{b}±34	2960^{a}±36	130	9.29*
Body weight after 6 weeks, g	3300^{b}±24	3600^{a}±26	300	21.43**
Body weight after 8 weeks, g	3600^{b}±33	3950^{a}±24	350	25.00**
Total gain, g (56 days)	950^{b}±14	1300^{a}±18	250	
Average daily gain, g/day	16.96^{b}±4	23.21^{a}±5	6.25	

a, b, means in the same column having different superscripts differ significantly.
** =significant at P<0.01, * = significant at P<0.05

Table 2. Effect of adding clove oil to the diet of mature male rabbits on bucks daily feed intake

Daily feed intake (DFI), g	Control	Clove oil	Change in feed intake
No of bucks	12	12	-
DFI of each buck during the first two week	165.0^{b}±8	175.5^{a}±10	+10.0*
DFI of each buck during the second two week	175.0^{b}±12	195.5^{a}±12	+20.0**.
DFI of each buck during the third two week	185.0^{b}±15	205.0^{a}±14	+20.0**
DFI of each buck during the fourth two week	195.0^{b}±13	220.0^{a}±21	+25.0**
Total feed intake for each buck , g/56 days	8640^{b}±35	9546^{a}±41	+906**
Average feed intake for each buck, g/daily	180.0^{b}±11	199.0^{a}±13	+19.0*

a, b Means in the same column having different superscripts differ significantly.
** =significant at P<0.01, * = significant at P<0.05

average feed intakes were optimized.

Our findings are consistent with a study by Iqbal *et al.* (2021), which discovered that clove oil taken orally once a day greatly improved rabbit growth performance and decreased the feed conversion ratio. When rabbits consumed clove oil, their feed intake dramatically increased. Clove oil may promote an increase in feed intake by making the diet more palatable (Anoh, 2020). El-Essawy *et al.* (2019) discovered that the dam ewes' daily body weight increase rate of their lambs till weaning was positively impacted by the clove essential oil supply throughout late pregnancy and lactation. The delivery of clove essential oil to the ewes resulted in enhanced total digestible nutrients and digested crude protein, according to the authors, as well as decreased nitrogen excretion through the feces and increased nitrogen retention. According to Kilic *et al.* (2011), eugenol in clove oil increased the effectiveness of utilizing nitrogen, as well as energy and protein, in the rumen. El-Kholy *et al.* (2022) discovered that clove oil injections considerably improved growth performance in rabbits and had a lasting impact on total body weight gain, feed intake, and feed conversion ratio. Clove powder treatment considerably enhanced live body weight, live body weight gain, and feed consumption in growing rabbits, according to Abdel-Azeem and Abd-El-Kader's findings from the year 2022. It also resulted in a considerable drop in the feed conversion ratio.

These outcomes could be attributed to clove oil's active ingredient, eugenol, which has been shown to promote digestion, boost rabbit performance, and possess antibacterial properties against intestinal bacteria as well as digestive enzyme activities in the intestinal mucosa and pancreas (Mehr *et al.*, 2014; El-Kholy *et al.*, 2022). Our findings of a considerable rise in the level of the hormones testosterone and immunoglobulin could be explained by an increase in feed intake and body weight growth in rabbits given clove oil (Mukhtar, 2011).

Effect of Clove Oil on Blood Protein Fractions

Table 3. Effect of clove oil in the diet of mature male rabbits on blood protein fractions

Protein fractions	Experimental weeks	Control	Clove oil	Change, %	P values & significant
Total protein (g/dl)	After two weeks	7.55[a]±0.05	6.13[b]±0.12	-18.81	P<0.01
	After four weeks	7.12[a]±0.02	6.15[b]±0.12	-13.62	P<0.01
	After six weeks	7.19[a]±0.04	5.93[b]±0.04	-17.52	P<0.01
	After eight weeks	7.18[a]±0.08	5.74[b]±0.04	-20.06	P<0.01
	Overall mean	7.26±0.098	5.99±0.096	-17.50	
Albumin (g/dl)	After two weeks	4.51[a]±0.03	3.57[b]±0.18	-20.84	P<0.01
	After four weeks	4.40[a]±0.07	3.58[b]±0.07	-18.64	P<0.01
	After six weeks	4.44[a]±0.05	3.45[b]±0.05	-22.30	P<0.01
	After eight weeks	4.40[a]±0.08	3.43[b]±0.11	-22.05	P<0.01
	Overall mean	4.44±0.03	3.51±0.04	-20.96	
γ-globulin (g/dl)	After two weeks	1.20[b]±0.02	1.62[a]±0.04	+35.00	P<0.01
	After four weeks	1.22[b]±0.01	1.54[a]±0.03	+26.23	P<0.01
	After six weeks	1.23[b]±0.03	1.54[a]±0.08	+25.20	P<0.01
	After eight weeks	1.21[b]±0.02	1.62[a]±0.03	+33.88	P<0.01
	Overall mean	1.22±0.01	1.58±0.02	+30.08	

[a, b] Means in the same column having different superscripts differ significantly.

The acquired findings for the effect of clove oil supplementation on blood protein fractions are reported in Table 3. After two, four, six, and eight weeks, the serum total protein in the clove oil group was reduced by 18.81, 13.62, 17.52, and 20.06%, respectively. Additionally, there was a decrease in the overall mean of total protein (5.99 g/dl) as a result of the treatment compared to the control (7.26 g/dl), with a reduction in percentage of almost 17.50%. This drop in total protein was consistent with an overall mean decrease of 20.96% and significant decreases in albumin values of 20.84, 18.64, 22.30, and 20.05% after two, four, six, and eight weeks, respectively. In contrast, after being treated with clove oil, levels of γ-globulin increased. The average was elevated by 30.08.

Clove supplementation caused a considerable decline in serum total protein in rabbits, and the overall mean was reduced by 17.50%. This fall in albumin values, which had a mean reduction of 20.96% overall, was consistent with a decrease in total protein that was also considerable. Contrarily, values of γ-globulin were increased by 30.08% after receiving clove oil therapy compared to the control group. El-Essawy *et al.* (2019) discovered that giving the sheep clove essential oil reduced their serum total protein levels considerably. El Gindy *et al.* (2021) discovered that giving clove oil to rabbits had no impact on their blood protein composition. But according to Abdel-Azeem and Abd-El-Kader (2022), supplementing with clove powder considerably raised the levels of plasma total protein, albumin, and globulin.

Because of variations in clove shape, supplement dose, and animal type, the reaction of clove to total proteins and albumin may differ. The amount of γ-globulin, on the other hand, increased significantly, which is consistent with the fact that feeding rabbit with clove oil activated their immune systems (Abdel-Azeem and Abd -El-Kader, 2022).

Effect of Clove Oil on Blood Cholesterol Fractions

The impact of clove oil on the cholesterol fractions in mature male rabbits is shown in Table 4. Low density lipoprotein (LDL), high density lipoprotein (HDL), and total cholesterol are all reduced by clove oil, with an overall mean decreased by 27.43% less than the control. The LDL was also reduced significantly, with an overall mean of 27.43%. On the other hand, there was a positive increase in serum HDL levels after six weeks of treatment (54.36%), eight weeks (41.46%), two weeks (39.54%), and lastly four weeks (35.22%), with a mean change percentage

Table 4. Effect of clove oil in the diet of mature male rabbits on blood cholesterol fraction

Cholesterol fractions	Experimental weeks	Control	Clove oil	Change, %	P values & significant
Total cholesterol (mg/dl)	After two weeks	47.66[a]±3.23	40.89[b]±1.78	-14.20	P<0.05
	After four weeks	50.84[a]±2.08	39.65[b]±4.33	-22.01	P<0.01
	After six weeks	53.30[a]±2.23	38.44b±1.50	-27.88	P<0.01
	After eight weeks	53.17[a]±2.40	36.14[b]±0.57	-32.03	P<0.01
	Overall mean	51.24±1.32	38.78±1.01	-24.03	
LDL (mg/dl)	After two weeks	19.53[a]±0.98	13.75[b]±1.20	-29.59	P<0.01
	After four weeks	18.00[a]±0.84	14.70[b]±0.25	-18.33	P<0.01
	After six weeks	19.66[a]±0.77	14.14[b]±0.18	-28.08	P<0.01
	After eight weeks	19.88[a]±0.83	13.18[b]±0.60	-33.70	P<0.01
	Overall mean	19.27±0.43	13.94±0.32	-27.43	
HDL (mg/dl)	After two weeks	6.50[b]±0.18	9.07[a]±0.28	+39.54	P<0.01
	After four weeks	6.90[b]±0.45	9.33[a]±0.23	+35.22	P<0.01
	After six weeks	6.31[b]±0.77	9.74[a]±0.37	+54.36	P<0.01
	After eight weeks	6.97[b]±0.46	9.86a±0.20	+41.46	P<0.01
	Overall mean	6.67±0.16	9.50±0.18	+42.65	

[a, b] Means in the same column having different superscripts differ significantly.

of 42.65% overall.

Adding clove oil to Buck's diet affects serum cholesterol fractions. The addition of clove oil to the meals of bucks resulted in a considerable reduction in total cholesterol and low density lipoprotein (LDL). On the other hand, serum high density lipoprotein (HDL) showed a positive improvement with a mean overall increase percentage of 42.65%.

The clove oil treatment resulted in a considerable reduction in cholesterol and triglyceride levels, according to Abdel-Azeem and Abd-El-Kader (2022) findings. Total cholesterol, total glycerides, high density lipoprotein and low density lipoprotein decreased significantly in rabbits injected with clove oil (El-Kholy *et al.* 2022). El-Essawy *et al.* (2019) discovered that giving clove essential oil to sheep considerably increased total lipids while dramatically increasing total cholesterol and triglycerides. These results could be linked to clove oil, whose active constituent (eugenol) has been found to stimulate the hypocholestrinum process.

Effect of Clove Oil on Kidney and Liver Functions

Regarding kidney function, the therapy had the desired effect of considerably reducing serum urea and creatinine levels at all study periods. Urea content decreased throughout the trial intervals in accordance with a 20.22% decline in the overall mean. Again, serum creatinine was reduced in relation to supplementing with clove oil. The superior significant decline was recorded after two weeks of treatment, and the total mean had decreased by 31.06%.

The clove oil treatment significantly reduced blood ALT levels, protecting the liver tissue. The overall mean decreased by 17.56% as a result. Additionally, at all trial intervals, serum AST decreased as a result of treatment; as a result, the overall mean has gotten worse by 19.14% (Table 5).

Since clove oil affects liver function, treating rabbits with it at every study interval caused a decrease in blood AST and ALT levels. Regarding kidney function, urea content decreased over the course of the experiment in step with a 20.22% decline in the overall mean. Again, serum creatinine was reduced in relation to supplementing with clove oil. To identify treatment-related harmful effects of the substance on the kidney in experimental animals, creatinine and urea concentrations are utilized as indicators of renal function.

These results show that the liver and renal functions are not adversely affected by the addition of clove oil. El-Essawy *et al.* (2019) demonstrated that supplementing ewes with clove essential oil resulted in normal renal function and

Table 5. Effect of clove oil in the diet of mature male rabbits on kidney and liver functions

Kidney & Liver function	Experimental weeks	Control	Clove oil	Change, %	P values & significant
Urea (mg/dl)	After two weeks	53.45[a]±1.33	45.77[b]±0.46	-14.37	P<0.05
	After four weeks	58.76[a]±1.14	44.67[b]±0.92	-23.98	P<0.01
	After six weeks	56.47[a]±2.09	43.65[b]±1.34	-22.70	P<0.01
	After eight weeks	56.68a±0.68	45.69[b]±1.62	-19.39	P<0.01
	Overall mean	56.34±1.09	44.95±0.50	-20.22	
Creatinine (mg/dl)	After two weeks	1.32[a]±0.06	0.89[b]±0.03	-32.58	P<0.01
	After four weeks	1.32[a]±0.06	0.93[b]±0.08	-29.55	P<0.01
	After six weeks	1.30[a]±0.02	0.88[b]±0.04	-32.31	P<0.01
	After eight weeks	1.32[a]±0.04	0.94[b]±0.09	-28.79	P<0.01
	Overall mean	1.32±0.005	0.91±0.015	-31.06	
ALT (U/l)	After two weeks	42.51[a]±1.95	36.66[b]±1.08	-13.76	P<0.05
	After four weeks	44.55[a]±0.63	33.20[b]±0.59	-25.48	P<0.01
	After six weeks	41.32[a]±1.38	34.20[b]±0.78	-17.23	P<0.05
	After eight weeks	40.66[a]±1.09	35.28[b]±1.05	-13.23	P<0.05
	Overall mean	42.26±0.85	34.84±0.74	-17.56	
AST (U/l)	After two weeks	39.32[a]±3.18	33.70[b]±1.76	-14.29	P<0.05
	After four weeks	39.57[a]±0.84	32.35[b]±0.74	-18.25	P<0.01
	After six weeks	38.83[a]±0.56	30.29[b]±0.64	-21.99	P<0.01
	After eight weeks	38.41[a]±0.39	29.88[b]±0.13	-22.21	P<0.01
	Overall mean	39.03±0.26	31.56±0.90	-19.14	

[a, b]Means in the same column having different superscripts differ significantly.

liver enzymes. Our results support those of Abdel-Azeem and Abd-El-Kader (2022), who observed that therapy with clove oil significantly reduced the activity of AST and ALT. After giving clove oil injections to rabbits, AST and ALT significantly decreased (El-Kholy *et al.*, 2022). Our findings, however, disagree with those of Omar (2003) and Tousson *et al.* (2011), who found that adding clove oil to rabbit diets significantly, increased blood ALT and AST activity.

The decrease in ALT and AST activity in bucks fed clove oil may be related to a change in the kind and quantity of amino acids that reached the small intestine of rabbits fed clove oil, according to a theory put forth by Tousson *et al.* (2011). Our results are consistent with those of El Gindy *et al.* (2021), who discovered that clove essential oil treatment reduced creatinine levels by 21.43% compared to control. But according to Tousson *et al.* (2011), the quantities of urea and creatinine in the rabbits' blood serum tended to increase significantly when clove oil-supplemented diets were contrasted with control diets (Tousson *et al.*, 2011). In general, clove oil and its active component, eugenol, can protect the kidneys from ischemic shock and other environmental toxins that could impair renal function (Hannan *et al.*, 2021).

Effect of Clove Oil on Antioxidant Enzymes Activity

Glutathione peroxidase (Gpx) and superoxide dismutase (SOD) levels were noticeably increased in the group that received clove oil treatment. The Gpx activity improved because of clove oil, and the total mean improvement was 21.53%. Clove oil also increased SOD, with an average increase of 28.88% (Table 6).

Glutathione peroxidase (Gpx) activity was improved by clove oil, and the overall mean improvement was 21.53%. Clove oil boosted superoxide dismutase (SOD) similarly, respectably increasing the total mean by 28.88%. It is evident that adding clove essential oil to the diet considerably increased the blood levels of antioxidants and may be employed in rabbit diets to boost antioxidant status.

Clove essential oil supplementation in the diet enhanced the blood concentration of antioxidant status (SOD and GPx) in growing rabbits, according to El Gindy *et al.* (2021). Male New Zealand rabbits' livers were used in the Mu'nisa

Table 6. Effect of clove oil in the diet of mature male rabbits on antioxidant enzymes activity

Serum antioxidant	Experimental weeks	Control	Clove oil	Change, %	P values & significant
Glutathione peroxidase (Gp_x), µmol/dl	After two weeks	25.53±0.67	29.90[a]±0.37	+17.12	P<0.05
	After four weeks	25.93±0.32	31.27[a]±0.27	+20.59	P<0.01
	After six weeks	26.50±0.45	32.17[a]±0.58	+21.40	P<0.01
	After eight weeks	26.83±0.23	34.00[a]±0.99	+26.72	P<0.01
	Overall mean	26.20±0.29	31.84±0.84	+21.53	
Superoxide dismutase (SOD), U/dl	After two weeks	10.70±0.32	13.47[a]±0.27	+25.89	P<0.01
	After four weeks	10.83±0.24	13.83[a]±0.42	+27.70	P<0.01
	After six weeks	11.07±0.58	14.47[a]±0.27	+30.71	P<0.01
	After eight weeks	11.43±0.58	15.00[a]±0.10	+31.23	P<0.01
	Overall mean	11.01±0.16	14.19±0.34	+28.88	

[a, b]Means in the same column having different superscripts differ significantly.

et al. (2015) study to examine the effect of Clove leaf methanol extract, and they discovered that the therapy boosted SOD activity.

Reactive oxygen species (ROS) and malondialdehyde (MDA) level rises associated with stress are what lead to oxidative stress. By up regulating antioxidant enzymes and compounds like GPx and SOD, clove oil, a natural antioxidant, may be able to reduce oxidative stress. As a result, ROS and MDA reduced (Hannan *et al.*, 2021).

Strong free radical scavenging activity in essential oils like clove oil, which can be employed to control free radicals, is explained by the synergistic interactions between phenolic components (Radünz *et al.* 2019). Antioxidants are essential for maintaining optimum health because they serve as the first line of defense against free radical damage. These newly created compounds interact with a variety of crucial molecules found in vital tissues; including lipids, proteins, and DNA, to create new chemicals that are harmful to DNA (Ercegovac *et al.* 2010).

Effect of Clove Oil on Testosterone Hormone and Glucose Concentrations

Clove oil treatment increased testosterone hormone levels by 66.53, 52.04, 64.15, and 56.35%, respectively, after two, four, six, and eight weeks, with a significant improvement in the overall mean of 59.77%. Contrarily, glucose levels decreased after treatment with clove oil, with a mean decrease of 18.23% (Table 7). Following treatment with clove oil, glucose levels decreased. Contrarily, testosterone hormone was increased by clove oil treatment, and the total means saw a significant improvement of 59.77%.

Our findings support the findings of Khan *et al.* (2006) that clove capsules can stimulate insulin activity and lower blood sugar levels in terms of glucose levels. Similarly, Abdel-Azeem and Abd-El-Kader (2022) discovered that treatment with clove oil caused levels of glucose to drop significantly.

The effects of some active clove compounds, such as dehydrodieugenol, which influence the different levels of the insulin signaling cascade pathway or post-insulin-receptor complex by increasing expression of glucose transporters and other molecular modulators of insulin activity, resulting in increased glucose intake by muscle cells, may be responsible for the observed decrease in blood glucose in the rabbit fed clove oil (Abdulrazak *et al.*, 2018).

Concerning with testosterone levels, clove oil had a favorable influence on the testosterone hormone in rabbits that were given injections of the substance (El-Kholy *et al.*, 2022). Clove may be helpful for increasing the production of gonadotropin-releasing hormone, according to the authors, who discovered that testosterone hormone levels were considerably increased in rabbits given clove injections.

The results of the study show that clove oil enhances the function of the prostate by increasing the level of testosterone, giving male rabbits

Table 7. Effect of clove oil in the diet of mature male rabbits on concentrations of testosterone hormone and glucose

Items	Experimental weeks	Control	Clove oil	Change, %	P values & significant
Testosterone (ng/ml)	After two weeks	5.02[b]±0.57	8.36[a]±0.57	+66.53	P<0.01
	After four weeks	5.88[b]±0.99	8.94[a]±0.61	+52.04	P<0.01
	After six weeks	6.22[b]±0.36	10.21[a]±0.72	+64.15	P<0.01
	After eight weeks	7.01[b]±0.58	10.96[a]±0.05	+56.35	P<0.01
	Overall mean	6.03±0.41	9.62±0.59	+59.77	
Glucose (mg/dl)	After two weeks	70.41[a]±3.21	59.73[b]±1.21	-15.17	P<0.05
	After four weeks	75.53[a]±2.28	60.55[b]±3.77	-19.83	P<0.05
	After six weeks	72.60[a]±2.83	60.47[b]±2.57	-16.71	P<0.05
	After eight weeks	74.43[a]±4.96	58.80[b]±1.66	-21.00	P<0.05
	Overall mean	73.24±1.12	59.89±0.41	-18.23	

[a, b] Means in the same column having different superscripts differ significantly.

the stamina and strength they require to perform the sexual act (Umar *et al.*, 2017). Because oil treatment had an effect on the hormone-producing enzyme 17-beta hydroxyl steroid dehydrogenase, oil-treated rabbits had increased testosterone levels. Clove oil has a biological effect on animals that raises levels of the hormone testosterone because of its antioxidant properties (Ayan *et al.*, 2015).

Effect of Clove Oil on Semen Properties

Table 8 and 9 provided information on the impact of clove oil on semen qualities. Due to the addition of clove oil, the reaction time was significantly decreased after two, four, six, and eight weeks by 11.36, 16.85, 18.18, and 13.41%, respectively. Reaction time (second-1) decreased overall by 10.91%. Clove oil increased ejaculate volume (x 10-2 ml) by 43.97% across all trial weeks as a percentage of the average oil, the reaction time was significantly decreased after two, four, six, and eight weeks by 11.36, 16.85, 18.18, and 13.41%, respectively. Reaction time (second-1) decreased overall by 10.91%. Clove oil increased ejaculate volume (x 10–2 ml) by 43.97% across all trial weeks as a percentage of the average. Once more, the percentage of total motility was significantly positively impacted by the treatment. After six weeks, it was found that the addition of clove oil considerably enhanced the total motility percentage, with the overall mean rising by 10.44%. The six-week period following the addition of clove oil at a significantly higher percentage had a significant impact on the progressive sperm motility (PMOT %), which improved as intended. The proportion of the overall mean showed a desired but accepted change of 11.86%. Through the beginning of the first week of the treatment, clove oil supplementation had no discernible impact on either the total motility percentage or the progressive sperm motility percentage (Table 8).

The effect of clove oil on the rabbit semen are shown in Table 9. Clove oil supplementation had no effect on the live sperm percentage during the first six weeks of the experiment, but after that, it considerably (11.34%) increased from 82.28 in the control group to 91.67% in the treatment group. The least amount of aberrant sperm was seen in male rabbits administered clove oil. Clove oil significantly decreased the proportion of dead sperm after the first two weeks of the experiment, going from 17.72% to 12.86%. These significant drops were consistent with a mean overall drop of 27.54%.

Following the first two weeks of the experiment, the therapy resulted in further appreciable increases in the percentage of normal sperm and decreases in abnormal sperm. The quantity of sperm cells per 106 ml-1 during intercourse and eight weeks after starting the medication increased significantly by 10.49 and 16.73%, respectively. During the four, six, and eight weeks of the experiment, the clove oil treatment significantly increased the total sperm yield (x106/ ejaculate) by 61.39, 116.04, and 164.38%, with

Table 8. Effect of clove oil in the diet of mature male rabbits on physical semen characteristics

Semen characteristics	Experimental weeks	Control	Clove oil	Change %	P values & significant
Reaction time second^{-1}	After two weeks	8.80^{a}±0.01	7.80^{b}±0.02	-11.36	P<0.05
	After four weeks	8.90^{a}±0.01	7.40^{b}±0.01	-16.85	P<0.05
	After six weeks	8.80^{a}±0.02	7.20^{b}±0.01	-18.18	P<0.05
	After eight weeks	8.20^{a}±0.01	7.10^{b}±0.01	-13.41	P<0.05
	Overall mean	8.68±0.16	7.38±0.15	-14.95	
Ejaculate volume x 10^{-2} ml	After two weeks	83.57^{b}±7.68	102.33^{a}±0.88	+22.45	P<0.01
	After four weeks	83.93^{b}±6.55	116.67^{a}±3.34	+39.01	P<0.01
	After six weeks	100.88^{b}±5.89	140.99^{a}±3.26	+39.76	P<0.01
	After eight weeks	106.88^{b}±5.50	186.67^{a}±8.83	+74.65	P<0.01
	Overall mean	93.82±5.94	136.67±18.48	+43.97	
Total motility percentage	After two weeks	81.88±2.82	87.67±0.33	+07.07	P>0.05
	After four weeks	82.79±1.40	90.67±0.67	+09.52	P>0.05
	After six weeks	82.66^{b}±1.38	92.87^{a}±0.66	+12.35	P<0.05
	After eight weeks	83.33^{b}±1.80	94.00^{a}±0.58	+12.80	P<0.05
	Overall mean	82.67±0.30	91.30±1.39	10.44	
Progressive sperm motility (PMOT, %)	After two weeks	68.30±1.80	71.67±0.88	+04.93	P>0.05
	After four weeks	69.67±1.36	74.33±0.67	+06.69	P>0.05
	After six weeks	69.54^{b}±1.65	80.66^{a}±1.01	+15.99	P<0.05
	After eight weeks	69.00^{b}±1.58	82.67^{a}±1.45	+19.81	P<0.05
	Overall mean	69.13±0.31	77.33±2.59	+11.86	

$^{a, b}$ Means in the same column having different superscripts differ significantly.

Table 9. Effect of adding clove oil to the diet of mature male rabbits on semen characteristics

Semen characteristics	Experimental weeks	Control	Clove oil	Change %	P values & significant
Live sperm (%)	After two weeks	81.67±0.88	81.67±0.88	00.00	-
	After four weeks	82.67±0.33	85.00±0.58	+02.82	P>0.05
	After six weeks	82.46±0.38	88.56±0.78	+07.40	P>0.05
	After eight weeks	82.33^{b}±1.20	91.67^{a}±0.67	+11.34	P<0.05
	Overall mean	82.28^{b}±0.22	86.73^{a}±2.17	+5.39	
Dead sperm (%)	After two weeks	18.33±0.88	16.67±0.88	-09.06	P>0.05
	After four weeks	17.33^{a}±0.33	15.00^{b}±0.58	-13.44	P<0.05
	After six weeks	17.54^{a}±0.38	11.44^{b}±0.78	-34.78	P<0.01
	After eight weeks	17.67^{a}±1.20	8.33^{b}±0.67	-52.86	P<0.01
	Overall mean	17.72±0.22	12.86±1.86	-27.54	
Normality %	After two weeks	66.67±2.03	65.33±1.20	-02.01	P>0.05
	After four weeks	67.00^{b}±2.08	77.67^{a}±1.45	+15.93	P<0.05
	After six weeks	67.49^{b}±2.06	77.30^{a}±1.87	+14.54	P<0.05
	After eight weeks	67.00^{b}±2.08	77.67^{a}±1.45	+15.93	P<0.05
	Overall mean	67.04±0.17	74.49±3.06	+11.10	
Sperm abnormalities (%)	After two weeks	35.33±2.03	34.67±1.20	-01.87	P>0.05
	After four weeks	35.00^{a}±2.08	30.67^{b}±1.45	-12.37	P<0.05
	After six weeks	32.51^{a}±2.06	22.70^{b}±1.87	-30.18	P<0.01
	After eight weeks	33.00^{a}±2.08	22.33^{b}±1.45	-32.33	P<0.01
	Overall mean	33.96±1.41	27.59±3.04	-19.19	
Sperm-cell concentration x10^{6} ml^{-1}	After two weeks	81.00±1.20	83.33±0.33	+02.88	P>0.05
	After four weeks	81.65±0.44	84.67±0.33	+03.70	P>0.05
	After six weeks	81.88^{b}±1.25	90.47^{a}±0.55	+10.49	P<0.05
	After eight weeks	81.67^{b}±0.88	95.33^{a}±0.88	+16.73	P<0.05
	Overall mean	81.55±0.19	88.45±2.77	+8.45	
Total sperms output (x10^{6} /ejaculate)	After two weeks	90.50±0.40	92.33±1.20	+02.02	P>0.05
	After four weeks	90.67^{b}±0.67	146.33^{a}±3.18	+61.39	P<0.01
	After six weeks	92.88^{b}±0.89	200.66^{a}±2.30	+116.04	P<0.01
	After eight weeks	92.67^{b}±1.45	245.00^{a}±5.01	+164.38	P<0.01
	Overall mean	91.68±0.63	171.08±33.11	+85.96	

$^{a, b}$ Means in the same column having different superscripts differ significantly.

an overall mean percentage increase in the total sperm yield of 85.96%.

It is evident that the group of bucks rabbits fed clove oil had considerably higher sperm motility, concentration, total count, total motile, total live, and total normal sperm per ejaculate than the control animals.

Clove oil considerably enhanced the qualities of rabbit bucks' semen. The use of clove oil significantly reduced the response time. The ejaculate volume, total sperm motility, progressive sperm motility, live sperm percentage, normal sperm percentage, number of sperm cells per 106 ml-1, and total sperm yield (x106/ejaculate) were all increased by clove oil, while the percentage of dead sperm and sperm abnormalities was decreased.

Utilizing clove oil as an antioxidant may lower oxidative stress and improve sperm motility (Castellini *et al.*, 2003; Castellini, 2008). Additionally, antioxidants play a crucial role in maintaining the spermatic cell membrane stability and avoiding the oxidation of sperm DNA and lipid peroxidation, which are both factors in male animal infertility and reactive oxygen species (Greco *et al.*, 2005). Baghshahi *et al.* (2014) looked at how the antioxidant properties of added clove bud extract affected the ram spermatozoa's semen quality measures. The scientists demonstrated that the sperm motility, progressive motility, viability, and movement characteristics of clove bud extract were all improved.

The semen quality in this study was improved because of clove oil's antioxidant properties. Clove may be helpful to increase lust and sperm quality by increasing the quantity of testosterone in the blood, which will ultimately increase sexual activity. Clove oil may have contributed to the improvement in sperm quality and quantity seen in the current study because it contains beneficial antioxidants that shield cells from oxidation and subsequently improve sperm concentration in rabbits.

CONCLUSION

In order to improve the quality of their semen and support more effective physiological activities, the study found that adding clove oil to the ration of mature male rabbits' (bucks) is a cheap and safe method that may be recommended for being used.

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