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IMPACT OF SUPPLEMENTATION WITH MILK THISTLE SEEDS AND ROSEMARY LEAVES ON SEMEN QUALITY, ANTIOXIDANTS STATUS AND REPRODUCTIVE PERFORMANCE OF RABBIT BUCKS

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ABSTRACT: This experiment aimed at investigating the effects of milk thistle seeds (MTS) and rosemary leaves (RL) at 5 and 10 g/kg diet as feed additives on semen quality, blood metabolites and reproductive performance on rabbit bucks. A total number of 35 male V-line rabbit bucks were distributed randomly into five experimental groups of 7 bucks each. The 1st group, which served as a control, did not supplement with MTS and RL in their basal diet. The 2nd and the 3rd groups were supplemented with MTS at 5 and 10 g/kg in their basal diet, respectively. The 4th and the 5th groups were fed the basal diet supplemented with RL at 5 and 10 g/kg, respectively. The best sperm concentration (SC), total sperm output (TSO), live sperm (LS), total live sperm (TLS) and total motile sperm (TMS) were obtained from bucks fed MTS at 10 g/kg diet followed by RL at 5 g/kg diet. Bucks received MTS 10g/kg diet significantly increased their blood serum testosterone compared to the control and this was associated with a significant increase in the fertility rate of the 10 g MTS group. In addition, RL at 5 g/kg significantly increased blood serum testosterone and fertility compared to the control, but the MTS group had the highest serum testosterone and fertility. In conclusion, MTS and RL at 10 and 5g/kg, respectively, significantly improved antioxidant status and liver markers, which led to a significant increase in semen quality and fertility in rabbit bucks.

Key words: Rabbits, Milk Thistle, Rosemary Leaves, Semen, Reproductive, Blood.

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INTRODUCTION

Oxidative stress (OS) has been considered as a major contributory factor to the infertility. Oxidative stress is the result of imbalance between the reactive oxygen species (ROS) and antioxidants in the body which can lead to sperm damage, deformity, and eventually male infertility. The term oxidative stress is generally applied when oxidants outnumber antioxidants (Du Plessis *et al*., 2008). An antioxidant that reduces oxidative stress and improves sperm motility could be useful in the management of male infertility (Bansal and Bilaspuri, 2008). Numerous antioxidants have proven beneficial in protecting damaging effects of ROS on sperm movement and against oxidative damage (Yousef *et al*., 2003). The male reproductive system is extremely sensitive to various environmental factors such as temperature, humidity, adverse weather conditions and contaminants such as drugs, pollution and toxins (Saradha *et al*., 2006; Attia *et al*., 2015). Mammalian spermatozoa membranes are rich in polyunsaturated fatty acids (PUFAs) and are sensitive to oxygen-induced damage mediated by lipid peroxidation, and thus are sensitive to reactive oxygen species (ROS) attack, which results in decreased sperm motility, presumably by a rapid loss of intracellular adenosine triphosphate (ATP). This increased axonemal damage while reduced viability of sperm, increased defects and impaired sperm capacitation and acrosome reaction (Bansal and Bilaspuri, 2007). Uncontrolled production of ROS that exceeds the antioxidant capacity of the seminal plasma leads to (OS), which is harmful to spermatozoa (Desai *et al*., 2010).The findings of Bansal and Bilaspuri. (2008) and Yousef *et al*. (2003) showed that antioxidants could be useful as a management tool for male infertility, and the antioxidant stability of semen can be enhanced by fortification of animal diets with antioxidant molecules (Attia and Kamel, 2012).

Phytogenic plants are a good source of antioxidants. Additionally, they are safe for both living organisms and the environment, but the phytogenic composition may varied widely due to method of processing, botanical origin, agronomic and environment factors (Windisch *et al*., 2008). Dorman *et al*. (2003) showed that some plants have been identified as sources of various phytochemicals, many of which possess powerful anti-oxidants among these plants is milk thistle (*Silybum marianum*, family: Compositae) (Wu *et al*., 2009). The active compound in milk thistle, derived from dried seeds, is silymarin, which represent approximately 4-6% of the milk thistle seed extract (Greenlee *et al*., 2007). The extract consists of about 65-80% silymarin (a flavonolignan complex) and 20-35% fatty acids, including linoleic acid (Kroll *et al*., 2007). Kshirsagar *et al*. (2013) and Suksomboon *et al*. (2011) indicated that silymarin acted as an excellent antioxidant, scavenging ROS and inhibiting lipid peroxidation, thereby protecting cells against OS. In addition, Ramadan *et al*. (2011) revealed that oral administration of milk thistle extract significantly decreased liver enzyme activity when given in repeated doses, and antioxidant enzymes were significantly increased in pre-treated extract of rat liver homogenate. They concluded that the inhibition percent of the reaction reactive rate by milk thistle extract in vitro confirms that it is a potent free radical scavenger.

Rosmarinus officinalis L. (common name, rosemary; family *Labiatae*) is known to be a rich source of active metabolites such as caffeic acid, and its derivatives, such as rosmarinic acid (Herrero *et al*., 2005). Ramirez *et al*. (2004) showed that rosmarinic acid has antioxidant effects and is well-absorbed in the gastrointestinal tract and from the skin and reduces the production of leukotriene B4 in human

polymorphonuclear leucocytes and inhibits the complement system. Furthermore, Harvàthová *et al*. (2010) and Isles *et al*. (2004) indicated that rosemary essential oil enriches rat hepatocytes' resistance against DNA-damaging oxidative agents and exhibits free radical-scavenging activity, as measured by DPPH assay. It has been proposed by Katerinopoulos *et al*. (2005) that rosemary and its constituents, especially caffeic acid derivatives such as rosmarinic acid, have therapeutic potential in the treatment of inflammatory diseases and hepatotoxicity. Rosemary is rich in phytochemical derivatives such as triterpenes, flavonoids and polyphenols. Carnosol, rosmanol and epirosmanol phenolic diterpenes of rosemary inhibit lipid peroxidation (Zeng and Wang, 2001). Moreover rosemary significantly attenuated the increase of lipid peroxidation and enhanced the levels of reduced glutathione and antioxidant enzyme activities in the kidney and testis in comparison to aspartame controls (Hozayen *et al*., 2014; Perez-Fons *et al*., 2006). The purpose of this study was to investigate the impact of milk thistle seeds and rosemary leaves as antioxidant diet supplements on semen quality, fertility, blood constituents and antioxidant profiles of rabbit bucks.

MATERIALS AND METHODS

Dried milk thistle seeds (MTS) and rosemary leaves (RL) were purchased from the local market and ground to a fine powder using an electric dry mill .The powder then stored in well-tied black plastic bags at room temperature (≈25° C) until used in the formulation of the bucks' diets. Total phenolic compounds (equivalent to Gallic acid) and antioxidant activity (equivalent to ascorbic acid) were determined according to the methods of Fogliano *et al*. (1999) and Viuda-Martos *et al*. (2010), respectively.

A total number of 35 male V-line rabbit bucks, initially aged 5 months old, with an average initial body weight of 2723 ±40.1 g, were used in this study during their 20th through 38th weeks of age. The animals were distributed randomly into five experimental groups of seven bucks each. The 1st group, which served as controls, did not supplemented with MTS and RL in their basal diet.The basal diet was composed of 10% maize, 13% barley, 3% molasses, 39.5% clover hay, 15% wheat bran, 17.5% soybean meal, 0.8% dicalcium phosphate, 0.5% limestone, 0.3% sodium chloride, 0.3% vitamin and mineral mixture and 0.1% methionine. The chemical compositions of the basal diet were analysed according to the AOAC (2007). The analysis showed that the compositions were 90.32% dry matter, 80.8% organic matter, 17.24% crude protein, 13.46% crude fibre, 2.8% ether extract, 9.52% ash and 56.98% nitrogen-free extract. The calculated digestible energy value was 2464 kcal/kg diet. The diet was formulated to meet the nutrient requirements of rabbit bucks according to NRC (1977). The 2nd and the 3rd groups were supplemented with MTS at 5 and 10 g/kg in the same basal diet, respectively. The 4th and the 5th group were fed the basal diet supplemented with RL at 5 and 10 g/kg, respectively. The rabbits were individually housed in galvanized Italian wire cages (30 × 25 × 40 cm) provided with feeders and automatic stainless steel nipple drinkers. The pelleted diet and fresh water were offered ad libitum. The pellets were 0.62 cm in length and 0.45 cm in diameter. The rabbits were kept under similar management (environmental temperature, humidity, stocking density, light-dark cycles and day lengths) and under similar hygienic conditions (with vaccinations and health care). The average temperature, relative humidity and the temperature–humidity index (THI) during the whole experimental period was 25.8C°, 67.7% and 28.1 respectively and 16:8 light-dark cycle. The diet was fed without antibiotics or coccidiostats. The temperature–humidity index (THI) was computed using the

formula established by Marai et al. (2001) for rabbits as follow: THI= db °C – {(0.31– 0.31 RH).(db °C –14.4)}
Where: db °C = dry bulb temperature in °C. RH= relative humidity expressed in percentage. The values obtained are then classified as follows:
THI<27.8= absence of heat stress.
27.8<THI<28.9= moderate heat stress.
28.9<THI<30.0= severe heat stress.
THI>30.0 very severe heat stress.

Semen was collected once weekly after 8 weeks of the initiation of experiment. Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT), initial hydrogen ion concentration (pH), ejaculate volume (EV), sperm concentration (SC), packed sperm volume (PSV), total sperm output (TSO), mass motility (MM), live sperm (LS), dead sperm (DS), abnormal sperm (AS), total motile sperm (TMS) and total live sperm (TLS).were measured according to (Smith and Mayer, 1955) and (Blom, 1950).

Five samples of blood per treatment were collected in the morning at 8 o'clock before regular time of feeding every 6 weeks (three times until the end of experiment) from an ear vein of the bucks and placed immediately in an ice tank. The samples were collected from the same animals after they had been selected randomly. The animals were colour-marked to easily identify them at the time of collection. The blood was collected in clean tubes with or without heparin to collect plasma and serum, respectively. Seminal plasma as well as blood plasma and serum were collected by centrifugation at 860 x g for 20 min at 4° C and stored at -60 C°. The phagocyte activity (PA), phagocyte index (PI) and lysosomal activity (LA) were determined according to Kawahara *et al*. (1991). Seminal plasma and serum metabolites such as total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) (U/L), serum total lipids, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low density low protein (VLDL), creatinine, urea, total antioxidant capacity (TAC), lipid peroxidation biomarkers such as malondialdehyde (MDA),were measured using commercial kits purchased from bio-diagnostic company (Recycling Crusher-SBM®, www.bio-diagnostic.com). Testosterone concentration in seminal plasma and blood serum were measured according to (Maruyama *et al*., 1987).

Fertility evaluation and the reproductive performance of bucks were done according to IRRG (2005). Bucks of each group were mated to 10 receptive nulliparous female rabbits. Litter size at birth (total and alive) was recorded for three consecutive parities. Other females replaced non- pregnant females to avoid or reduce female problems.

Statistical analysis

Data were subjected to statistical analyses using the GLM procedure of statistical analysis software (SAS) version 6.11 (SAS, 1996). All percentages were log transformed (log10 x + 1) to normalize data distribution. The mean difference at $P \leq 0.05$ was tested using the Student-Newman-Keuls test. Chi square analysis was used to evaluate the effect of experimental treatments on mortality.

RESULTS AND DISCUSSION

The results in Table 1 indicate that MTS had greater total polyphenols (124.4%) and consequently more antioxidant activity (38.1%) than that of rosemary leaves. This indicates that MTS had a great potential as antioxidant agent than RL. These results are in agreement with those observed by Kim *et al*. (2011) who reported that phenolic substances have been shown to be responsible for the antioxidant activity of plant materials. In addition, higher antioxidant activity has been positively correlated with the

concentration of phenolic compounds in extracts (Sun *et al*., 2007).

The semen quality of bucks fed diet supplemented with MTS and RL are shown in Table 2. There were significant effects ($p<0.0001$) of treatments on RT, PH, EV, SC, PSV, TSO, MM, LS, DS, AS, TMS, TLS, fertility (%), litter size and live kits per litter. The results demonstrated that the superiority of semen quality of the groups supplemented with MTS at 10 g/kg. The groups supplemented with RL at 10 g/kg diet showed the lowest semen quality and fertility. However, this group recorded the highest EV. At birth, litter size and live kits per litter were significantly greater of different groups on MTS and RL than those of the control group. These results along with increased fertility indicate an improvement in reproductive efficiency of bucks due to phytogenic supplementations (Windisch *et al*., 2008).

Data in Table 3 reveals the impact of MTS and RL supplementations on seminal plasma antioxidant status and biochemical constituents (ALT, AST, AST/ALT ratio, total protein, albumin, globulin, and albumin/globulin) and serum and seminal plasma testosterone levels of bucks rabbits. It was found that RL at 5 g/kg resulted in significantly greater TAC compared to control and RL at 10 g/kg in the diet groups however, differences between RL at 5 g/kg group and MTS groups were not significant. There was no significant difference in seminal plasma MDA due to the experimental treatments. All additives significantly reduced seminal plasma ALT compared to the control group. Addition of RL at 5 g/kg in the diet was the most effective dose, followed by the same dose of MTS. Seminal plasma AST significantly reduced by MTS treatment at 10 g/kg in the diet compared to the other experimental groups. Addition of MTS and RL in the diet significantly increased seminal plasma AST/ALT ratio except for group supplemented with MTS at 10 g/kg in the diet compared to control. There was an absence of other effects on the seminal plasma albumin, globulin and the albumin/globulin ratio.

The blood serum and seminal plasma testosterone levels of bucks fed diet supplemented with MTS and RL are shown in (Table 3).There were a significant increase in serum testosterone level of the groups supplemented with MTS and RL except for group supplemented with RL at 10 g/kg in the diet compared to control group. In addition, group supplemented with MTS at 5 g/ kg diet significantly increased seminal plasma testosterone level compared to the 10 g dose of RL. The lowest serum testosterone level was from the control group and that whose diet was supplemented with RL at 10 g/kg.

These results demonstrated the superiority of the semen quality and serum testosterone of groups supplemented with MTS at 10 g/kg, but the same dose of RL induced the lowest semen quality, serum testosterone and fertility, although this group recorded the highest EV. The differences between the two groups of 10 g/kg of MTS and RL in fertility can be explained by the differences in serum and seminal plasma testosterone concentrations. It was observed that semen quality, testosterone and the fertility of rabbit bucks depend on the type and level of supplemented herbs. The enhancing effects of MTS on the semen quality of the bucks are in accordance with findings of the studies reported by Malekinejad *et al*. (2012) and Vigay Kranti *et al*. (2013), and could be attributed to the effects of antioxidants (Attia and Kamel, 2012). Silymarin plays a crucial role in counteracting the strong toxicopathological footprints left by doxorubicin and its metabolites (Malekinejad *et al*., 2012; Vigay Kranti *et al*., 2013). This is the reason behind the ability of silymarin to maintain spermatogenesis and to help the sperm perform a successful fertilization. In addition to the antioxidant property of silibinin that inhibits radical formation,

binds some radical species, interferes with lipid peroxidation of membranes, and increases the intracellular content of scavengers (Verschoyle *et al*., 2008). In addition, silymarin-treated animals were protected from varicocele-induced testicular atrophy, and these animals showed a significant ($P < 0.05$) increase in the percentage of seminiferous tubules with positive tubular differentiation, repopulation, and spermatogenic indices (Moshtaghion *et al*., 2013). Furthermore, silymarin improved the varicocele-induced carbohydrate reduction in germinal cells and silymarin extracted from *Silybum marianum* caused an improvement in some semen qualities and in the quantity of male gonadal hormones in rabbits supplemented with nickel chloride (Abid Ali *et al*., 2015). The latter authors reported also that, silibinin in doses of 100 mg/kg BW or 150 mg/kg BW produced a significant increase ($P<0.05$) in testosterone compared to a control group, which agrees with the boosting influence of MTS on serum and seminal plasma testosterone found herein. Furthermore, MTS was found to contain greater polyphenols and antioxidant activity than that of RL. On the other hand, silibinin in doses of 50, 100 or 150 mg/kg BW for mice had no significant ($P>0.05$) effects on the percent of motile sperms, dead/live sperms and abnormal sperms in comparison to negative controls (Oufi *et al*., 2012).

The present investigation showed that RL supplementation at 5 g/kg of diet (equivalent to 20 mg/kg BW) caused a significant increase in semen characteristics, while a contrary effect was observed in groups supplemented with RL at 10 g/kg of diet (equivalent to 40 mg/kg BW). Thus RL can enhance the reproductive function of bucks' rabbits when given at 5g/kg diet. This was associated with the greatest TAC of this group. Rosemary was found also to be a good source of polyphenols and consequently antioxidant activity, but to less extent than MTS (Table 1). In the research literature, the effect of RL (*Rosmarinus officinalis)* on the semen characteristics of rabbit bucks is inconclusive. For example, the findings of Nusier *et al*. (2007) showed that RL extract at 250 and 500 mg/kg BW caused a significant decrease in the germinal cell population. In addition, Heidari-Vala *et al*. (2013) indicated that sperm motility decreased, though not significantly, in treated groups in comparison to controls. Yet the motility and viability of sperm concurrently declined following increasing doses of *Rosmarinus officinalis* extract at 50 and 100 mg/kg BW in male rats. On the contrary, Superchi *et al*. (2005) revealed that rosemary extract at low doses (12.5 ppm) in the diet of boars resulted in an increase in the SC ($P=<0.01$) and LS percentage ($P<0.05$). Also, the serum testosterone concentration was higher when compared to controls during the summer season. They suggested that the antioxidant activity of rosemary extract could limit the negative effects of high temperatures on the reproductive efficiency of boars. Similarly, Purohit and Daradka (1999) showed that a significant increase in the SM of cauda epididymis was observed in groups supplemented with rosemary extract. In addition, rosmarinic acid at the low concentration of 20 mg/kg BW had the ability in rats to increase serum testosterone and sexual behaviour, such as ejaculation, mounts and lordosis in comparison to other groups (Farzadi *et al*., 2011). Aspartame rats treated with rosemary extract produced a significant increase ($P < 0.05$) in serum FSH levels compared to the unsupplemented group, but the changes ($P>0.05$) in LH and testosterone levels were insignificant (Hozayen *et al*., 2014).

Data in Table 4 illustrates the effect of MTS and/or RL supplementations on the liver and renal functions of rabbit bucks. The data show that MTS at 10 g/kg and RL at 5 and 10 g/kg in the diet respectively

significantly decreased blood serum ALT compared to the control group and MTS 5g/kg group. The groups on RL supplementations significantly decreased serum AST compared to the control group. In addition, group supplemented with RL at 5 g/kg significantly decreased serum AST compared to the MTS groups however, differences between RL 10g/kg group and MTS groups were not significant. Addition of MTS at 10 g/kg in the diet was decreased significantly serum urea compared to control and RL treatments. In addition, supplementation with MTS at 5 g/kg decreased significantly serum creatinine compared to control, but these groups did not significantly different form other groups. There was an absence of other effects on the blood serum urea/creatinine ratio and serum total protein, albumin, globulin and the albumin/globulin ratio.

Table 5 displays the effects of different treatments on TAC, MDA, glucose, total lipids, total cholesterol, LDL, VLDL, HDL and triglycerides in blood serum for rabbit bucks. The results show MTS at 10 g/kg in the diet supplementations significantly increased blood serum TAC compared to control and RL at 5 g/kg groups. Addition of MTS in the diet significantly decreased blood serum MDA compared to control group, but difference between groups on MTS and RL was not significant. The lowest and the highest concentration of serum LDL- and HDL-cholesterol, respectively were from group on 10 g/kg MTS. Serum glucose, total lipids, triglycerides, total cholesterol and VLDL-cholesterol were not significantly affected by phytogenic supplementations.

The present results indicated that different levels of MTS and RL are safe and might improve liver and renal functions. This could be attributed to the antioxidant capacity of MTS and RL. It is generally assumed that the antioxidant molecules from rosemary may act as free radical scavengers, but might play an additional role by regulating the activity and/or expression of certain enzymatic systems implicated in relevant physiological processes such as apoptosis, tumour promotion and intracellular signal transduction. Similarly, rosemary significantly enhanced glutathione and antioxidant enzyme activities in kidneys and testes compared to aspartame controls (Hozayen *et al*., 2014 and Perez-Fons *et al*., 2006). They also observed an almost normal histological architecture of the kidneys in the treated groups compared to the aspartame controls. Similarly, oral administration of milk thistle extract significantly decreased liver enzyme activity when given in repeated doses and increased the antioxidant enzymes, showing that milk thistle extract is a potent free radical scavenger (Ramadan *et al*., 2011). Also, ethanol extract of milk thistle significantly decreased liver enzymes after carbon tetrachloride (CCL4) exposure, and noticed some equal improvements in the histopathological studies for the protective groups with the extract (Kim *et al*., 2009; Shaker *et al*., 2010 and Ramadan *et al*., 2011) and rosemary essential oil enriched hepatocyte resistance to oxidative damage and exhibited free radical-scavenging activity (Harvàthová *et al*., 2010).

The increase in TAC of group on 10 g MTS was connected with the lowest and the highest concentrations of serum LDL- and HDL-cholesterol, respectively. In this regard, Kreeman *et al*. (1998) indicated that silymarin in milk thistle given to rats with diet-induced hypercholesterolemia demonstrated an anticholesterolemic effect as an increase in HDL cholesterol and a decrease in total and biliary cholesterol. In addition, Suksomboon *et al*. (2011) showed that milk thistle, with its antioxidant actions, might benefit people at risk of high cholesterol and diabetes. Similarly, flavonoids of milk thistle (*Silybum marianum*) had potent antioxidant effects (Muriel *et al*., 1990; Lawrence *et al*., 2000

and Ramadan *et al*., 2011) as indicated by significant increases of superoxide anions and lipid oxygen radicals due to lipid peroxidation (Muriel *et al*., 1990 and Shaker *et al*., 2010). The latter authors demonstrated in vivo that the antioxidant activity of milk thistle is via increasing the glutathione, which is an important antioxidant that detoxifies an array of hormones, drugs and chemicals. Silymarin was found also to increase superoxide dismutase in cell cultures. The abovementioned researchers revealed the potential of MTS as an antioxidant and as an antioxidant and a cholesterol-lowering agent.

Table 6 demonstrate no significant effects for MTS and RL on RBCs characteristics as well as on RBCs and its fractions. The lack of significant effects of MTS and RL on RBCs characteristics and most of the WBCs as general health indices and its fractions indicated that MTS and RL are safe feed additives for rabbit bucks. Similarly, milk thistle modulates the imbalance between cell survival and apoptosis through interference with the expressions of cell cycle regulators and proteins involved in apoptosis, as well as anti-inflammatory, anti-metastatic, and chemo-/radio-protective effects (Hogan *et al*., 2007; Ramasamy *et al*., 2008).

It can be observed in Table 7 that there were no effects of MTS and RL on the immune indices of the buck rabbits, as PA, lysozyme activity and IgA were not significantly affected. Whereas, eosinophil increased significantly in buck rabbits fed diet supplemented with 10g/kg of MTS compared to control and rosemary groups. However, PI was significantly affected by treatments, but mean differences among different experimental groups were not significant as results of high standard division. Supplementation with MTS at 5 g/kg significantly increased IgG compared to control group. It is interesting to report that RL at 5 g/kg significantly decreased IgM compared to the MTS at 5 g/kg. Immunoglobulin M is of vital importance in complement activation and agglutination. Immunoglobulin M is predominantly found in the lymph fluid and blood, and is a very effective neutralizing agent in the early stages of disease. The increase in the IgM can be a sign of recent infection or of exposure to antigens and the effects of rosemary on leukocyte migration highlight an important mechanism of the anti-inflammatory action of rosemary (Wiersma *et al*., 1998 and Noqueira de Melo *et al*., 2011). In addition, Aghazadeh (2011) revealed that the anti-apoptotic and anti-inflammatory properties of milk thistle in the treatment of steatohepatitis (fatty liver) in rats and histopathological examinations showed that the crude extract of milk thistle reduced the severity of non-alcoholic steatohepatitis (NASH).

In conclusion, MTS and RL at 10 and 5g/ kg respectively enhanced the antioxidant status, liver functions, semen quality and Fertility of rabbit bucks.

Table (1): Average ±SE of total polyphenols (equivalent to Gallic acid) and antioxidant activity (equivalent to ascorbic acid)

Samples	Total polyphenols (equivalent to Gallic acid)	Antioxidant activity (equivalent to ascorbic acid)
Milk thistle seed (mg/ 100 g)	392.1±5.6	780±84.9
Rosemary leaves (mg/100 g)	174.7±9.5	565±21.2

Table(2): Effect of milk thistle seeds and rosemary leaves on semen quality and fertility of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	Traits														
		RT (SC)	pH	EV (ml)	SC (10^6)	PSV (%)	TSO (10^6/ejaculate)	MM (%)	LS (%)	DS (%)	AS (%)	TMS (10^6)	TLS ($X10^6$)	Fertility (%)	Litter size (kits/litter at birth)	Live kits/litter at birth)
Control	0	16.0^{a}	7.80^{a}	0.88^{c}	454^{c}	14.2^{b}	403^{d}	69.8^{c}	72.7^{c}	10.0^{a}	17.3^{a}	284^{d}	296^{d}	83.6^{d}	6.84^{b}	6.36^{b}
Milk thistle seeds	5	12.8^{b}	7.67^{b}	0.97^{a}	486^{b}	14.3^{ab}	475^{b}	75.3^{ab}	79.5^{b}	7.3^{bc}	13.1^{bc}	360^{b}	382^{b}	90.0^{b}	7.41^{a}	7.07^{a}
	10	12.1^{c}	7.66^{b}	0.98^{a}	528^{a}	14.5^{a}	520^{a}	76.3^{a}	81.1^{a}	6.6^{c}	12.3^{c}	399^{a}	424^{a}	93.6^{a}	7.50^{a}	7.17^{a}
Rosemary leaves	5	11.3^{d}	7.63^{b}	0.93^{b}	482^{b}	14.5^{a}	450^{c}	76.1^{a}	79.7^{b}	7.0^{bc}	13.3^{bc}	341^{c}	359^{c}	87.5^{c}	7.63^{a}	7.42^{a}
	10	12.6^{b}	7.70^{b}	1.0^{a}	445^{c}	14.2^{b}	444^{c}	74.3^{b}	78.6^{b}	7.6^{b}	13.8^{b}	330^{c}	349^{c}	80.0^{e}	7.61^{a}	7.11^{a}
Statistical analysis																
Treatments		VHS	VHS	VHS	VHS	VHS	VHS	VHS	VHS	HS	VHS	S	S	S	S	S
RMSE		1.41	0.27	0.10	43.8	0.572	64.4	3.71	3.12	2.13	2.73	50.5	50.3	3.67	0.84	0.80

^{a,b,c,d}- values within a column with different letters are significantly different (P≤0.05, significant (S), highly significant (HS), very highly significant (VHS).
Reaction time (RT), ejaculate volume (EV), sperm concentration (SC), packed sperm volume (PSV), total sperm output (TSO), mass motility (MM), live sperm (LS), dead sperm (DS), abnormal sperm (AS), total motile sperm (TMS), total live sperm (TLS).

Table (3): Effect of milk thistle seeds and rosemary leaves on seminal plasma biochemical constituents of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	antioxidant constituents		enzymes			Total protein (g/dI)	Alb (g/dL)	Glb (g/dL)	Alb /glb ratio	Testosterone, (ng/ml)	
		TAC (mm/L)	MDA (mm/L)	ALT (IU)	AST (IU)	AST/ ALT					Serum	Seminal plasma
Control	0	0.97^{b}	3.67	34.2^{a}	54.1^{a}	1.59^{d}	5.72	2.78	2.94	0.95	0.668^{b}	0.976^{ab}
Milk thistle seeds	5	1.23^{ab}	3.65	28.2^{c}	54.6^{a}	1.94^{b}	5.52	2.71	2.81	0.99	0.799^{a}	0.992^{a}
	10	1.23^{ab}	3.62	30.8^{b}	49.9^{b}	1.62^{d}	5.36	2.71	2.65	1.05	0.810^{a}	0.960^{ab}
Rosemary leaves	5	1.60^{a}	3.59	24.9^{d}	53.2^{a}	2.14^{a}	5.30	2.67	2.63	1.03	0.767^{a}	0.926^{ab}
	10	1.05^{b}	3.74	$30.\ 9^{b}$	54.5^{a}	1.77^{c}	5.88	3.09	2.79	1.12	0.693^{b}	0.905^{b}
Statistical analysis												
Treatments		HS	NS	VHS	HS	VHS	NS	NS	NS	NS	VHS	S
RMSE		0.279	0.238	1.17	2.16	0.091	0.37	0.32	0.31	0.20	0.028	0.043

^{a,b,c,d}- values within a column with different letters are significantly different ($P \leq 0.05$), significant (S), highly significant (HS), very highly significant (VHS).; not significant (NS). Total antioxidant capacity (TAC), MAD malnodialdehyde (MDA), alanine amino transferase (ALT), aspartate amino transferase (AST), albumin (Alb), globulin (Glb).

Table(4): Effect of milk thistle seeds and rosemary leaves on liver and renal functions in blood serum of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	Liver function			Kidney function			Total protein (g/dI)	Alb (g/dL)	Glb (g/dL)	Alb/glb ratio
		ALT (IU)	AST (IU)	AST/ ALT	Urea (mg/dl)	Creatinine (mg/dl)	Urea/ Creatinine				
Control	0	36. 7^{a}	58.0^{a}	1.59^{ab}	44.8^{a}	1.49^{a}	30.2	5.34	2.53	2.81	0.94
Milk thistle	5	35.6^{a}	54.4^{ab}	1.53^{b}	42.1^{ab}	1.26^{b}	33.8	5.50	2.58	2.92	0.90
seeds	10	31.1^{b}	55.6^{ab}	1.79^{a}	40.0^{b}	1.36^{ab}	29.7	5.60	2.65	2.95	0.92
Rosemary	5	28.0^{c}	49.4^{c}	1.78^{ab}	44.7^{a}	1.37^{ab}	32.8	5.60	2.61	2.99	0.88
leaves	10	30.2^{bc}	53.3^{b}	1.77^{ab}	45.2^{a}	1.44^{ab}	31.5	5.60	2.64	2.96	0.93
Statistical analysis											
Treatments		VHS	VHS	S	HS	S	NS	NS	NS	NS	NS
RMSE		1.81	2.69	0.134	2.60	0.115	3.44	0.27	0.29	0.400	0.994

$^{a,b,c-}$ values within a column with different letters are significantly different (P≤0.05), significant (S), highly significant (HS), very highly significant (VHS)., not significant (NS). Alanine amino transferase (ALT), aspartate amino transferase (AST), albumin (Alb), globulin (glb).

Table (5): Effect of milk thistle seeds and rosemary leaves on antioxidants indices and blood biochemical constituents of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	antioxidant constituents		Glucose (g/dl)	Total lipid (mg/dL)	Total cholesterol (mg/dl)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dl)
		TAC (mm/L)	MDA (mm/L)							
Control	0	1.27^{b}	3.44^{a}	87.3	339	128	71.8^{a}	26.1	30.3^{b}	131
Milk thistle seeds	5	1.64^{ab}	3.03^{b}	78.6	338	126	67.8^{ab}	26.4	32.0^{b}	132
	10	1.99^{a}	2.95^{b}	81.5	334	128	65.6^{b}	25.5	36.5^{a}	127
Rosemary leaves	5	1.36^{b}	3.13^{ab}	85.7	337	130	72.4^{a}	26.6	31.2^{b}	133
	10	1.54^{ab}	3.32^{ab}	84.9	332	129	72.4^{a}	26.1	31.0^{b}	131
Statistical analysis										
Treatments		HS	HS	NS	NS	NS	HS	NS	HS	NS
RMSE		0.307	0.23١	6.20	9.9	4.30	3.45	1.42	2.87	7.12

$^{a,b-}$ values within a column with different letters are significantly different (P≤0.05), highly significant (HS), not significant (NS). Total antioxidant capacity (TAC) malnodialdehyde (MDA), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL).

Table (6): Effect of milk thistle seeds and rosemary leaves on red blood cells traits of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	Traits					
		RBC ($\times 10^6$/mL)	PCV (%)	Hgb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)
Control	0	5.6	40.4	9.7	73.0	17. 5	24.0
Milk thistle seeds	5	6.2	41.0	9.8	66.7	15.8	23.7
	10	6.1	41.4	9.9	68.7	16.4	23.9
Rosemary leaves	5	6.2	40.9	10.3	66.3	16.7	25.4
	10	6.2	41.7	10.0	67.9	16.3	24.0
Statistical analysis							
Treatments		NS	NS	NS	NS	NS	NS
RMSE		0.78	1.66	0.85	8.05	1.81	1.72

Not significant (NS). Red blood cell counts (RBC), paced cells volume (PCV), hemoglobin (Hgb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC).

Table (7): Effect of milk thistle seeds and rosemary leaves on white blood cell characteristics and immune indices of rabbit bucks (least squares means ± RMSE).

Treatments	Dose g/kg	Traits											
		WBC ($\times 10^3$/mL)	Lym (10^3)	Mon (10^3)	Bas (10^3)	Eos (10^3)	Net (10^3)	PA	PI	LysA	IgA	IgG	IgM
Control	0	5.36	42.9	12.9	0.5	11.9^{bc}	31.8	21.4	1.90	0.110	77.2	974^{b}	243^{ab}
Milk thistle seeds	5	5.24	42.7	12.0	0.7	12.0^{ab}	32.6	21.0	1.70	0.114	81.0	985^{a}	245^{a}
	10	5.29	41.8	11.4	0.6	12.9^{a}	33.3	21.4	1.98	0.090	79.4	980^{ab}	242^{ab}
Rosemary leaves	5	5.15	42.5	12.6	0.7	11.7^{b}	33.1	21.2	1.90	0.118	78.2	980^{ab}	239^{b}
	10	5.22	42.0	11.9	0.7	11.5^{b}	33.9	21.0	1.72	0.118	79.6	982^{ab}	243^{ab}
Statistical analysis													
Treatments		NS	NS	NS	NS	S	NS	NS	S	NS	NS	S	HS
RMSE		0.54	1.63	1.33	0.50	1.00	2.86	1.21	0.16	0.02	2.01	4.90	2.65

$^{a\text{-}b\text{-}c}$-values within a column with different letters are significantly different ($P \leq 0.05$), significant (S), highly significant (HS), not significant (NS). White blood cell (WBC), lymphocytes (Lym), monocytes (Mon), Basophils (Bas), eosinophil (Eos), neutrophils (Net), Phagocytic activity (PA), Phagoytic index (PI), lysozyme activity(Lys.A), immunoglobulin type A (IgA), immunoglobulin type G (IgG), immunoglobulin type M (IgM).

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الملخص العربى

تأثير إضافة بذور شوك اللبن و أوراق إكليل الجبل إلى علائق ذكور الأرانب على صفات جودة السائل المنوي و مضادات الاكسدة و الأداء التناسلي

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أجريت هذه التجربة لتقييم تأثير بذور شوك اللبن وأوراق إكليل الجبل بالمستويات ٥ ، ١٠ جم /كجم فى عليقة ذكور الارانب على صفات السائل المنوي وصفات الدم وتاثيرهم على الاداء التناسلي والفسيولوجي و المناعي. استخدم في هذه الدراسة ٣٥ ذكر من سلالة الفي لاين عمر ٥ شهور تم توزيعها عشوائيا على خمس معاملات ٧ ذكور في كل معاملة ، المجموعة الاولى كونترول تتغذى على العليقة بدون أي إضافات المجموعة الثانية والثالثة تم اضافة ٥ ، ١٠ جم/ كجم من بذور شوك اللبن على الترتيب والمجموعة الرابعة والخامسة تم اضافة ٥ ، ١٠ جم / كجم من أوراق إكليل الجبل على الترتيب.

وأوضحت النتائج أن أفضل تركيز للحيوانات المنوية وكذا أفضل نسبة حي وأفضل حركة تقدمية للحيوانات المنوية كانت للمجموعة المعاملة بِـ ١٠ جم / كجم من بذور شوك اللبن فى العلف تلتها المجموعة المعاملة بـ ٥ جم / كجم من أوراق إكليل الجبل.

إضافة بذور شوك اللبن عند مستوى ١٠ جم / كجم فى العلف حسنت معنويا مدلولات مضادات الاكسدة ووظائف الكبد تلتها المجموعة المعاملة بـ ٥ جم / كجم من أوراق إكليل الجبل بالمقارنة بمجموعة الكنترول.

كما أوضحت النتائج زيادة معنوية في تركيز هرمون التستوستيرون فى سيرم الدم للمجموعة المضاف اليها ١٠ جم / كجم من بذور شوك اللبن بالمقارنة بمجموعة الكنترول كما ارتبط ذلك بارتفاع معنوى لنسبة الخصوبة في نفس المجموعة بالمقارنة بمجموعة الكنترول. بالإضافة إلى ذلك فإن إضافة أوراق إكليل الجبل بنسبة ٥ جم / كجم فى علف الأرانب ادى كذلك إلى زيادة معنوية فى نسبة هرمون التستوستيرون فى سيرم الدم للذكور وكذلك زيادة معنوية لنسبة الخصوبة في ذات المجموعة بالمقارنة بمجموعة الكنترول ،ولكن تاثير إضافة ١٠ جم / كجم من بذور شوك اللبن كان اقوي معنويا بالمقارنة بمجموعة أوراق إكليل الجبل.

الخلاصة

استخدام بذور شوك اللبن وأوراق إكليل الجبل عند مستوى ١٠ ، ٥ جم /كجم على الترتيب فى علائق ذكور الارانب البالغة حسنت معنويا مدلولات مضادات الاكسدة ووظائف الكبد مما أدى إلى تحسن معنوى فى صفات السائل المنوي وبالتالى تحسن معنوى في نسبة الخصوبة.