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Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves

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A R T I C L E I N F O

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A B S T R A C T

The study aimed to investigate the effects of milk thistle seeds (MTS) and rosemary leaves (RL) both at 5 and 10 g/kg diet on reproductive performance, semen quality and blood metabolites of rabbit bucks. A total of 35 rabbit bucks were randomly distributed into five experimental groups (7 bucks/group). All the groups were fed the same basal diet. The 1st group (control) did not have MTS and RL in its basal diet. The 2nd and 3rd groups were supplemented with MTS at 5 and 10 g/kg, respectively. The 4th and 5th groups were fed the basal diet supplemented with RL at 5 and 10 g/kg, respectively. The sperm concentration (SC), total sperm output (TSO), live sperm (LS), total live sperm (TLS) and total motile sperm (TMS) were significantly greater in the bucks fed MTS at 10 and RL at 5 g/kg diet than the control group. Bucks fed MTS at 10 g/kg diet had higher fertility than the control. Also, RL 5 g/kg group showed higher testosterone and fertility than the control, but the MTS 10 g/kg group showed the highest value for both parameters. In conclusion, MTS and RL at 10 and 5 g/kg, respectively, significantly improved the semen quality and the fertility and MTS also increased the economic efficiency of rabbit bucks.

1. Introduction

Presently, there is a great interest in using phytogenic antioxidants as productive enhancers and oxidative stress (OS) controller (Attia et al., 2011; Attia and Kamel, 2012) and these can improve the sperm motility and count (Wirleitner et al., 2012). However, the phytogenic composition of plants can vary widely due to the botanical origin, method of processing, agronomic and environment factors (Windisch et al., 2008). 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) attacks, which reduce the sperm viability, increase defects and impair the sperm capacitation and acrosome reaction (Bansal and Bilaspuri, 2007). An excessive production of ROS, exceeding the antioxidant capacity of the seminal plasma, leads to the oxidative stress, which is harmful to spermatozoa (Desai et al., 2010). Thus, antioxidants could be useful as a management tool to improve male fertility, and it has been reported that the antioxidant stability of semen can be enhanced by fortification of animal diets with antioxidant molecules (Yousef et al., 2003; Bansal and Bilaspuri, 2008).

Dorman et al. (2003) reported that some plants have been identified as sources of various phytochemicals, many of which possess

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anti-oxidant power. These plants include milk thistle (*Silybum marianum*, family *Compositae*) (Wu et al., 2009). The active compound in milk thistle is silymarin which is found in dried seeds and represents 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). Silymarin has been shown to act as an excellent antioxidant, scavenging ROS and inhibiting lipid peroxidation, thereby protecting cells against OS (Suksomboon et al., 2011; Kshirsagar et al., 2013). ***Rosmarinus officinalis*** L. (common name, rosemary; family *Labiatae*) is another phytogenic plant, rich in active metabolites such as caffeic acid and rosmarinic acid (Herrero et al., 2005). Rosmarinic acid has antioxidant effects and is absorbed through both the gastrointestinal tract and the skin.

The oral administration of milk thistle extracts significantly decreased the liver enzyme activity when given in repeated doses, and antioxidant enzymes were significantly increased in pre-treated extract of rat liver homogenate, confirming that milk thistle is a potent free radical scavenger (Ramadan et al., 2011).

The rosmarinic acid reduces the production of leukotriene B_4 in human polymorphonuclear leucocytes and inhibits the complement system (Ramirez et al., 2004). In rats, the rosemary essential oil improves the resistance of hepatocytes against DNA-damaging oxidative agents and exhibits a free radical-scavenging activity (Isles et al., 2004; Harvàthová et al., 2010). The rosemary and its constituents, especially the caffeic acid and its derivatives such as the rosmarinic acid, have positive effects in the treatment of inflammatory diseases and hepatotoxicosis (Katerinopoulos et al., 2005). The rosemary is also 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). The rosemary significantly attenuates the increase of lipid peroxidation and enhances the levels of reduced glutathione and antioxidant enzyme activities in the kidney and testis when compared to aspartame (Perez-Fons et al., 2006; Hozayen et al., 2014). Hence the purpose of this study was to investigate the semen quality, antioxidant status and reproductive performance of rabbit bucks fed milk thistle seeds and rosemary leaves as antioxidant diet supplements.

2. Material and methods

2.1. Tested supplements

Dried milk thistle seeds and rosemary leaves were purchased from a local market and grounded into a fine powder by an electric dry mill; the powders were stored in well-tied black plastic bags at room temperature (≈25° C) until the inclusion in the diets of the bucks. The total phenolic compounds (equivalent to gallic acid) and the antioxidant activity (equivalent to ascorbic acid) were determined according to the methods proposed by Fogliano et al. (1999) and Viuda-Martos et al. (2010), respectively.

2.2. Animals and experimental design

A total of 35, twenty weeks old, V-line rabbit bucks (average initial body weight of 2723 g ± 40.1) were used in this study. The animals were randomly distributed into five experimental groups (7 bucks/group). All the groups were fed the same basal diet, formulated to meet the nutrient requirements of rabbit bucks according to NRC (1977). NRC (1977) equations were used to calculate the digestible energy content of the diet. Ingredients and chemical-nutritional characteristics of the diet (AOAC, 2007) are reported in Table 1. The diet included no antibiotics or coccidiostatics. Table 2 shows the content of polyphenols and the antioxidant activity of milk thistle and rosemary seeds.

Between 20 and 38 weeks of age, the groups were submitted to the following dietary treatments: the 1st group (control) was not supplemented with MTS and RL; the 2nd and the 3rd groups were supplemented with MTS at 5 and 10 g/kg basal diet, respectively; the 4th and 5th group were fed the basal diet supplemented with RL at 5 and 10 g/kg, respectively.

Table 1
Ingredients and chemical-nutritional characteristics of the basal diet.

Ingredients	g/kg	Chemical-nutritional characteristics	
Clover hay	395	Dry matter, %	90.3
Soybean meal 44%	175	Crude Protein, %	17.2
Wheat bran	150	Crude Fibre, %	13.5
Barley	130	Ether extract, %	2.8
Maize	100	Ash, %	9.5
Molasses	30	Digestible Energy, Kcal/kg	2464
Dicalcium Phosphate	8		
Limestone	5		
Sodium chloride	3		
Min-Vit*	3		
Methionine	1		

*Provides per kg of diet: Vit. A 6000 IU; Vit. D 450 IU; Vit.E 40 mg; Vit. K 1 mg; Vit. B1 1 mg; Vit. B2 3 mg; Vit. B3180 mg; Vit. B6 39 mg; Folic acid 2.5 mg; Vit. B12 5 µg; Pantothenic acid 10 mg; Biotin 10 µg; Choline Chloride 1200 mg; Zn 35 mg; Fe 38 mg; Cu 5 mg; I 0.2 mg; Se 0.05 mg and Mn 15 mg.