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Effects of *Tinospora cordifolia* supplementation on semen quality and hormonal profile in rams

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ABSTRACT

The present study was undertaken to assess the effect of dietary supplementation of *Tinospora cordifolia* on physico-morphological, biochemical, antioxidant profiles and serum testosterone concentration in Muzzafarnagari rams. Twelve rams were randomly divided into two groups, control ($n = 6$) and supplemental ($n = 6$) group. The control group was fed with a diet satisfying NRC recommendations whereas the supplemental group was fed with *T. cordifolia* at the rate of 1 g/kg body weight for 6 months. The semen samples were collected 60 days post-feeding. The result revealed that *T. cordifolia* supplementation did not have a significant effect on physico-morphological, biochemical attributes of semen and serum testosterone concentrations in rams. The concentration of cholesterol, superoxide dismutase (SOD) and catalase were, however, increased ($P < 0.05$) in seminal plasma. It was concluded that the possible protective effects of *T. cordifolia* supplementation were enhancing antioxidant enzymes and cholesterol concentrations in semen which may be protected the spermatozoa during cryopreservation and thus enhancing fertility in farm animals.

1. Introduction

Artificial insemination plays an important role in the genetic improvement through which a single ejaculate is used to impregnate many females. The process of semen preservation has attained a status of industry in dairy husbandry because of wide use, but as yet has not attained a similar amount of use in small ruminants. This is attributed to the fact that during preservation, large numbers of sperm are rendered incapable of fertilizing the ovum because these processes exert physiological as well as chemical stress on the sperm membrane associated with oxidative stress induced by the free radicals (Chatterjee et al., 2001). Because the sperm membrane contains greater amounts of polyunsaturated fatty acids and lacks significant antioxidant content in the cytoplasm, sperm are highly susceptible to lipid peroxidation (Sinha et al., 1996) and this can be overcome by incorporation of antioxidants in diluents (Perumal et al., 2011) and/or feeding of antioxidants to the animals (Ziegler and Filer, 1996).

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to be used as remedies for humankind and animals. Several authors have reviewed the beneficial uses of these plant

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species (Speroni and Scartezzini, 2000; Matkowski, 2008) as an antioxidant to improve fertility. Similarly some plants have antifertility effects such as *Gossypium herbaceum* (Hoffer et al., 1987) and *Azadirachta indica* (Khillare and Shrivastav, 2003).

T. cordifolia is basically an immuno-modulator (Nair et al., 2004) and also has anti-cancer (Singh et al., 2005), nerve cell protecting (Rawal et al., 2004), anti-diabetic, blood cholesterol-lowering (Stanley et al., 2003) and liver-protective (Bishayi et al., 2002) actions. *T. cordifolia* has a wide variety of antioxidant properties and reduces reactive oxygen and nitrogen species (ROS/RNS; Veena et al., 2002). During photo-sensitization, *T. cordifolia* prevents lipid peroxidation and protects the activities of antioxidant enzymes such as SOD and catalase. *T. cordifolia* attenuates oxidative stress mediated cell injury and exerts antioxidant effects in the cytosol as well as on gene expression (Rawal et al., 2004).

There is scarcity of information on the effect of *T. cordifolia* supplementation on semen quality in domestic animals as an antioxidant. The present study was, therefore, undertaken to explore the effect of *T. cordifolia* supplementation on semen quality, biochemical profiles of semen and serum testosterone concentrations in rams.

2. Materials and methods

2.1. Experimental animals

The present study was conducted on 12 Muzzafarnagari rams of 1.5–2 years of age with good body condition (score 5–6 on a 10 point scale) and average body weight of 38.3 ± 1.23 kg maintained at Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The rams were maintained as per the standard criteria fixed for maintenance of breeding rams during the entire course of investigation.

The rams were randomly divided into two groups having six rams in each group. All these animals were fed with standard basal diet consisting of concentrate mixture and roughage (wheat straw) to meet their nutrient requirements according to NRC (1985) throughout the study period. Semen and blood was collected from the animals 2 months post-feeding.

2.2. Diet formulation and T. cordifolia supplementation

Formulation of diet and supplementation of *T. cordifolia* were according to body weight and total dry matter intake. All the animals were offered concentrate at 1% of body weight and wheat straw for ad libitum consumption. The dietary regimen of the two groups was as follows:

Group I (Control): Basal diet alone and Group II (Supplemented): Basal diet + *T. cordifolia* at 1 g/kg body weight.

The calculated quantity of *T. cordifolia* whole plant powder was mixed uniformly with the concentrate mixture. The concentrate was fed to the rams individually so as to ensure consumption at 1 g/kg body weight. The mean daily intake (g/day) of concentrate and *T. cordifolia* irrespective of the dietary treatments were 345.64 ± 11.60 and 39.20 ± 2.70, respectively.

2.3. Collection of semen

The semen was collected once a week from all the rams for 6 weeks with use of an artificial vagina (AV). The collections were obtained by exposing the ram to the oestrous ewe. Immediately after collection, the tubes containing semen were placed in a small thermo-flask having water at 37 °C for further processing. A total of six ejaculates from each ram were collected for assessment; overall 72 ejaculates were collected and assessed. After routine semen evaluation, the semen was divided into two parts, of which one part was centrifuged immediately at 4000 rpm for 20 min at 4 °C to harvest the seminal plasma. The seminal plasma and sperm pellets were stored at −20 °C for the further processing and analysis.

2.4. Semen analysis

The seminal parameters such as volume, colour, pH, mass activity, individual motility, concentration (heamocytometer), livability (Eosin–Nigrosin stain), abnormality (Rose Bengal stain), acrosomal (Giemsa stain) and plasma membrane integrity (based on hypo-osmotic swelling test, HOST, Jeyendran et al., 1984) were assessed. All the six ejaculates collected from each animal were averaged for statistical analysis.

2.5. Biochemical assessment of seminal plasma

The biochemical profiles from seminal plasma were assessed using commercially available Span diagnostic kits (Span Diagnostic Limited, Surat, India). The biochemical profiles were aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (AKP), acid phosphatase (ACP), total cholesterol and total protein (Lowry et al., 1951).

2.6. Estimation of enzymatic activity

2.6.1. Estimation of SOD activity

The SOD activity of the seminal plasma was estimated using the method as described by Madesh and Balasubramanian (1997) with some modifications. The reaction mixture consisted of 100 μl seminal plasma, 60 μl 1.25 mM MTT, 1280 μl PBS and 15 μl 1 mM pyrogallol. Pyrogallol solution was prepared freshly every time to prevent auto oxidation. In blank, enzyme/seminal plasma was replaced with same amount of PBS. The reaction of formation of formazan crystals by reduction of MTT was terminated by addition of 1.5 ml DMSO and the readings were taken spectrophotometrically at 570 nm using Double beam UV–VIS Spectrophotometer (DBS; Model-UV5704SS, ECIL, India). One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%. The total SOD activity was expressed in units per mg of protein present in seminal plasma.