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Effect of monensin supplementation on semen quality and seminal antioxidants of alpine beetal crossbred bucks

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Abstract
The present study was conducted to study the effect of monensin supplementation on semen quality and seminal antioxidants status of alpine beetal crossbred bucks. Ten sexually mature bucks were divided into two groups. The control animals were fed according to the requirement of ICAR (2013) standards, with 40:60 ratios of concentrate and green roughage. The treatment group was fed similar to control with addition of 20 mg/head/d of monensin sodium for three months. Weekly semen samples were collected for three months i.e twelve ejaculates. Mean± SE value of sperm concentration (106/ml) was 2655.83±69.39 and 2708±62.54, progressive motility 79.7±0.91 and 80.3±0.88, acrosomal integrity 85.28±0.34 and 86.33±0.46 and HOST 80.11±0.51 and 81.15±0.47 in control and treatment group respectively. Mean± SE value of superoxide dismutase (SOD) 150.72±3.3 and 151.90±3.1, Melondialdihyde (MDA) 0.72±0.018 and 0.71±0.009 and glutathione peroxidase (GPx) 10.36±0.12 and 10.50±0.08 in control and treatment group respectively. Monensin (20 mg/head/d) had no significant (P>0.05) effect on semen quality parameters and antioxidant status of seminal plasma.

Keywords: monensin, superoxide dismutase, glutathione peroxidase, acrosomal integrity, progressive motility

Introduction
Artificial vagina method was used to collect the semen samples. Sperm Concentration was assayed as per Kale (1995). Haemocytometer (Neubauer's chamber) set was used for estimation of sperm concentration. The hypo-osmotic swelling test was performed to check the integrity of plasma membrane. It was performed as described by Correa *et al.* (1994) [1]. For assessment of individual motility, semen was diluted with Tris. 100 µl of undiluted semen was mixed with 900 µl of Tris buffer in a pre-warmed sterilized tube. The percentage of progressive motile spermatozoa was observed under microscope (20x). The method of Hancock (1952) [2] was followed for staining to measure acrosomal integrity.
To study the effect of monensin supplementation on antioxidant status and lipid peroxidation in seminal plasma in sexually mature bucks. Seminal plasma was separated from the collected semen samples. Semen was centrifuged at 700xG for 15 min to separate seminal plasma. After separation, the seminal plasma was stored at -20 °C for further analyses. Seminal plasma Glutathione peroxidase (GPX) and Melondialdihyde (MDA) were estimated in seminal plasma samples in Goat by using ELISA Kit supplied by Bioassay Technology, 1713 Junjilng International Building, 218 Ningguo Rd. Yangpu Dist. SH. China. One-way analysis of variance was used for the comparison among means. The significance of differences was determined by the Tukey's multiple range tests. Significance was determined at P<0.05.

Results and Discussion
1. Semen quality
Mean± SE value of semen quality parameters is presented in table 4.27. Mean± SE value of sperm concentration (10^6/ml) in control and treatment group was 2655.83±69.39 and 2708±62.54 respectively. Mean± SE value of progressive motility in control and treatment group was 79.7±0.91 and 80.3±0.88 respectively. Mean and SE value of acrosomal integrity in control and treatment group was 85.28±0.34 and 86.33±0.46 respectively. Mean and SE value of HOST in control and treatment group was 80.11±0.51 and 81.15±0.47 respectively. There was no significant difference found in above semen quality between control and treatment group. It was reported no effect of monensin on semen quality in yearling bulls (Peters *et al.*

1994) [4]. Similarly Downs *et al.,* (2000) [3] reported the effect of laidlomycin propionate (ionophore) on semen quality and they didn't found any significant effect. Khairi *et al.,* (2019) [5] reported slight enhancement of semen quality in monensin treated rams which was due to potential mechanism involved in the monensin-mediated increase in LH secretion at the level of the Golgi apparatus of endocrine cells. *In vitro* studies suggested that monensin was capable of redirecting hormone production towards secretion rather than storage in perfused rat pituitary fragments (Farmer *et al*. 1989). It was concluded that monensin supplementation had no detrimental effect on semen quality.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are important antioxidant enzymes of the seminal plasma, which scavenge the free radicals and convert them into stable products. Decreased concentration of SOD and GPx associated with poor sperm quality and reproductive performance (Yue *et al*. 2010). Mean± SE value of superoxide dismutase (SOD), Melondialdihyde (MDA) and glutathione peroxidase (GPx) is presented in table 1. Mean± SE value of superoxide dismutase (IU/ml) in control and treatment group was 150.72±3.3 and 151.90±3.1 respectively. There was no significant difference between control and treatment groups. Mean± SE value of glutathione peroxidase (mIU/ml) in control and treatment group was 10.36±0.12 and 10.50±0.08 respectively. There was no significant difference between control and treatment groups. Sperm membrane predominantly consists of unsaturated fatty acid which is more susceptible for oxidative damage. Melondialdihyde (MDA) is the major breakdown product of lipid peroxidation and their estimation in seminal plasma reveals the status of lipid peroxidation. Mean± SE value of MDA (µmol/ml) in seminal plasma of control and treatment was 0.72±0.018 and 0.71±0.009 respectively. There was no significant difference between control and treatment groups. The results reveal that monensin did not change the seminal plasma antioxidants enzymes or degree of lipid peroxidation. It is concluded that monensin does not have any detrimental effect on semen. However, no literature is available to support the present findings. Table 1 Scrotum circumference (cm) of bucks during monensin supplementation.

Table 1: Effect of monensin supplementation on semen quality in post pubertal bucks

Parameters	Control	Treatment
Sperm concentration (X 10^6 /ml)	2655.83±69.39	2708±62.54
Progressive motility (%)	79.7±0.91	80.3±0.88
HOST (%)	80.11±0.51	81.15±0.47
Acrosomal integrity (%)	85.28±0.34	86.33±0.46

Table 2: Effect of monensin supplementation on antioxidant status in seminal plasma

Antioxidants	Control	Treatment
Superoxide dismutase (IU/ml)	150.72±3.3	151.90±3.1
Glutathione peroxidase (mIU/ml)	10.36±0.12	10.50±0.08
MDA (µmol/ml)	0.72±0.018	0.71±0.009

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