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Effect of Organic Zinc Supplementation on Semen Production and Quality in Kankrej Bulls

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ABSTRACT

Background: Zinc is found in very high concentration in semen and male reproductive tract and acts as a hormone receptor modulator for testosterone production in Leydig cells. Thus, it plays a very important role in male reproduction. Moreover, Indian soils are very deficient in zinc as per earlier reports and recent studies have shown that supplementation of zinc has improved qualitative as well as the quantitative aspects of semen quality in bulls. So, a study has been planned to study the effect of organic zinc supplementation on semen production and quality in kankrej bulls.

Methods: A total of 8 healthy Kankrej bulls under semen collection were selected for the study. Basal diet (concentrate mixture, green and dry fodder) was fed during pre-supplementation and zinc propionate @ 100 ppm/bull was supplemented to the basal diet during post-supplementation for a period of 60 days to complete one spermatogenic cycle. A total of 128 ejaculates (64 pre-supplementation and 64 post-supplementation) were collected.

Result: Mean semen volume was non-significant (P>0.05) between the treatment groups. Mean sperm concentration was 1325.68±80.08 and 2162.36±112.0 million/ml pre- and post-supplementation, respectively. Feeding of zinc propionate significantly (P<0.01) increased semen sperm concentration. The average mass motility of sperms was significantly (P<0.01) higher during post-supplementation (3.92%) than the pre-supplementation (3.78%). The overall live sperms were significantly (P<0.01) higher during post-supplementation (73.33±0.91%) than the pre-supplementation (63.53±1.03%). It may be concluded that supplementation of 100 ppm of zinc propionate in the diet of Kankrej bulls improved semen quality in terms of quantitative and qualitative characteristics of semen.

Key words: Kankrej bull, Organic Zinc, Semen quality.

INTRODUCTION

Zinc is an essential micro-mineral for animal life with the concentration of 20-50 mg/ kg body weight in livestock. It has a wide range of biological functions. Zinc is involved in cell replication and differentiation, particularly in nucleic acid metabolism throughout the life. It is also responsible for production, storage and secretion of hormones, maintenance of immune system and electrolyte balance (Mcdonald *et al.,* 2007). It is found in high concentration in the male reproductive tract as well as in semen (Chia *et al.*, 2000) and plays a very important role in male fertility as it acts as hormone receptor modulator for testosterone in Leydig cell leads to maintenance of sperm production and quality (Roy *et al*., 2013; Kaliky *et al.,* 2019). Zn deficiency affects the DNA synthesis in the germ cell, sperm formation and also reduce the sperm motility (Yamaguchi *et al*., 2009). Forty eight percent of Indian soils are deficient in Zn (Arunachalam *et al*., 2013) which may affect the male reproduction. Thus, Zinc deficiency is one of the major factors for infertility in breeding bulls. Kankrej is proud native breed of Banaskantha region of Gujarat. Semen producing ability and quality of individual bull is highly essentials to ensure the supply of superior quality germplasm for maintaining the production as well as the conservation of indigenous breeds. Recent studies also have supported the fact that supplementation of zinc has improved qualitative as well as the quantitative aspects of semen quality in bulls (Thakur *et al*., 2019; Majumder *et al*., 2020). So, a study was undertaken to study the effect of organic zinc supplementation on semen parameters in Kankrej bulls.

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MATERIALS AND METHODS

The use of the animals and the experimental procedure in present trial was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the Article 13 of the rules of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), laid down by the Government of India. The IAEC approval number was VETCOLL/IAEC/2019/14/PROTOCOL-06.

Location of study, experimental animals and feeding

An experiment was carried out at Livestock Research Station of Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Banaskantha, Gujarat (Latitude 24.35° N, longitude 72.59° E and Mean Sea level l89). Eight Kankrej bulls (Age 4-6 years) under semen collection were selected as experimental animals. A total of 128 ejaculates (64 pre-supplementation and 64 post-supplementation) were collected from the bulls. They were fed on basal diet during pre-supplementation period, while Zinc Propionate @ 100 ppm/animal was supplemented to basal diet as a source of zinc (KemTRACE Zn; Kemin Industries) during post-supplementation for a period of 60 days. Dose of 100 ppm was selected by taking into consideration weight of bulls and previous records of experiments. The animals were fed as per ICAR (2013) feeding standards to meet the nutrient requirements. The data of dry matter intake of the bulls during experimental period is given in Table 3. All the bulls were kept in a well-ventilated shed having a cemented floor and arrangements for individual feeding. Clean and fresh drinking water was provided twice daily *i.e*., at 9.00 am and 2.00 pm to all the animals.

Proximate composition of feeds and fodder

Representative sample of feeds and fodder were analyzed (AOAC, 1999) for proximate composition. Zinc (ppm) for different feedstuffs was calculated using standard reference values.

Collection of semen and evaluation of its quantitative and qualitative characteristics

The semen from all the bulls was collected in an artificial vagina over a dummy or male partner for initial pre-supplementation period of two months followed by two months of experimental zinc feeding. Then, 8 ejaculates from each animal were collected to study the effect of Zn supplementation on semen characteristics. Two months feeding was carried out intentionally to complete one cycle of spermatogenesis (for sperm formation and maturation) which is 60 days in bulls. All semen samples were evaluated for quantitative and qualitative characteristics.

Evaluation of semen quantitative characteristics

Ejaculate volume (mL) of semen was recorded to the nearest 0.1 mL in a graduated glass tube. The concentration of sperm (millions/ml) in the fresh semen was determined using a photometer (Accucel, IMV, France) against 530 nM wave length.

Evaluation of semen qualitative characteristics

Mass motility of semen was graded from a 0-5 scale, based on the appearance of waves and swirls created by sperm movement when visualized by keeping one drop of semen on a glass slide, without cover slip, under low power microscopic magnification (10×) (Salisbury *et al.*,1978). Extremely rapid waves or swirl motion of sperms were given a scale of 5, comparatively slower waves and swirls were given a 4 numerical scale and likewise further slow moving, extremely slow moving, no movement and non-motile sperm were given 3, 2, 1 and 0 numerical scales, respectively. The individual motility of freshly diluted semen was assessed after covering a semen drop on a glass slide with a thin cover slip at 37°C, under high power magnification (40×). Live sperm percentage was calculated by using Eosin-Nigrosin stain. The same stain was utilized for counting abnormal sperm percentage.

Statistical analysis

The data were analyzed for statistical significance using “paired- t test” (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Proximate composition of feeds and fodders

The proximate composition of feeds and fodders fed to the experimental animals is given in Table 1. Nutrient and of concentrate (BIS Type II), oat forage and jowar hay was within the range as reported by Joshi *et al*, 2021. Zinc (ppm) content of different feeds was also calculated and was found to be within normal range (Chaudhary *et al*., 2017). Dry matter intake Average dry matter intake/ 100 kg body weight of experimental animals pre and post supplementation was 2.46 and 2.54.

Semen quantitative attributes

The average semen quantitative and qualitative attributes of Kankrej bulls are presented in Table 2. Overall mean values for semen volume were 3.99±0.19 and 3.81±0.16 ml per ejaculate in pre- and post-supplementation, respectively, the difference was non-significant (P>0.05) among groups. However, mean values for sperm concentration were significantly (P<0.05) higher in post supplementation group (2162.36±112.0 million/ml vs 1325.68±80.08) as compared to pre-supplementation groups. Zinc has an indispensable role in male spermatogenesis. The production of any cell including sperm necessitates extensive mitotic or meiotic cell division which requires DNA polymerase and RNA polymerase. Both of these are Zn dependent enzymes for their activity. Zn also helps in encoding a transcription factor involved in spermatogenesis and stimulates production of testosterone from seminiferous tubules. Zinc containing

Table 1: Chemical composition (% DM basis) of feeds and fodders fed to Kankrej bulls.

Composition	Concentrate feed	Oat forage	Jowar hay
Dry matter	94.91	17.44	11.83
Crude protein	19.57	8.92	6.39
Crude fibre	6.58	27.67	32.25
Ether extract	4.88	3.12	1.34
Ash	8.02	7.34	9.07
NFE	60.95	52.95	50.95
Zinc (ppm)*	60.24	21.50	54.00

*Calculated values.

Table 2: Average semen quantitative and qualitative attributes of Kankrej bulls.

Attributes	Pre-supplementation	Post-supplementation	P value
Volume (ml)	3.99±0.19	3.81±0.16	0.480
Sperm concentration (Millions/ml)	1325.68^{a}±80.08	2162.36^{b}±112.0	P<0.01
Mass motility (%)	3.78^{a}±0.03	3.92^{b}±0.02	P<0.01
Initial motility (%)	76.08^{a}±0.70	78.33^{b}±0.41	0.004
Live sperms (%)	63.53^{a}±1.03	73.33^{b}±0.91	P<0.01
Abnormal sperms (%)	6.83^{b}±0.58	5.35^{a}±0.45	0.040
Post-thaw motility (%)	46.17^{a}±1.40	51.14^{b}±0.86	P<0.01

Table 3: Total dry matter intake of Kankrej bulls during the experimental period.

Attributes	Pre-supplementation	Post-supplementation	P value
Average dry matter intake/ 100 kg body weight	2.46±0.02	2.54±0.08	0.860

metalloenzymes like sorbitol dehydrogenase and lactate dehydrogenase are also very important enzymes involved in the process of spermatogenesis (Kumar *et al*., 2006). All these factors may account for improved sperm concentration and sperm number per ejaculate in Zn-supplemented group.

Semen qualitative attributes

The average semen qualitative attributes of Kankrej bulls are presented in Table 2. The average mass motility of sperms was significantly (P<0.01) higher during post-supplementation (3.92±0.02%) when compared to the pre-supplementation (3.78±0.03%). Mean initial motility of sperms was 76.08±0.70 and 78.33±0.41% in pre- and post-supplementation, respectively, the difference being significant (P<0.05). The overall live sperms were significantly (P<0.01) higher during post-supplementation (73.33±0.91%) when compared to the pre-supplementation (63.53±1.03%). The average abnormal sperms were significantly (P<0.01) reduced during post-supplementation (5.35±0.45%) than the pre-supplementation (6.83±0.58%). Mean post-thaw motility of sperms were significantly (P<0.01) higher during post-supplementation (51.14 ±0.86%) as compared to the pre-supplementation (46.17±1.40%).

Flagella help in the sperm motility which in turns depends on energy supply in the form of ATP. Zinc helps in energy utilization of sperm through stimulation of ATP cleavage by prostasomes in semen (Ronquist *et al*., 2013). Zn is also a scavenger of free oxygen radical and protects sperm from oxidative damage and lipid peroxidation by inhibiting phospholipase (Kumar *et al*., 2006). Kumar *et al*. (2006) found increased live spermatozoa (%) in Zn supplemented group in crossbred bulls. Sabhapati *et al*. (2015) also observed increased live spermatozoa (%) in Zn supplemented group in Karan Fries during supplemented period.

CONCLUSION

It may be concluded that additional supplementation of 100 ppm of zinc propionate in the diet of Kankrej bulls improved semen quality in terms of quantitative and qualitative characteristics.

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Conflict of interests

There is no conflict of interest.

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