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Effect of Dietary Supplementation of Trace Minerals on Semen Production Performance of Sahiwal Bulls During Winter Season

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ABSTRACT

Present study was planned to evaluate the effect of supplemental trace minerals on semen production performance of Sahiwal bulls. Twelve Sahiwal bulls were grouped as T_1, T_2 and T_3 and were supplemented with 0, 1.5 and 2.0 times trace minerals over and above the basal diet in T_1, T_2 and T_3, respectively for a study period of 120 days. All the animals were fed to meet their nutrient requirements as per ICAR (2013). Semen collection was done twice a week in all the groups and samples were evaluated fortnightly for quantitative and qualitative attributes. A digestion trial of seven- days collection period was conducted during d 90-96 of experimental feeding to assess the digestibility of nutrients. Activity of alkaline phosphatase (ALP), lipid peroxidation (LPO), blood antioxidant status and concentrations of (Zn, Cu, Mn, Co, Fe, Ca and P) were estimated in blood and seminal plasma. It was found that additional trace minerals supplementation had no influence on body weight, dry matter intake and digestibility of nutrients among the groups. All seminal parameters except mass activity and post-thaw motility were higher ($P<0.05$) in T_3 group in comparison to T_1 and T_2 groups in fresh as well as frozen semen. Blood antioxidant status was improved in T_3 group. A reduction ($P<0.05$) in LPO activity in fresh and frozen semen in T_3 group was observed. It may be concluded that dietary supplementation of inorganic Zn, Cu, Mn and Co (40 ppm Zn, 10 ppm Cu, 15ppm Mn and 0.11ppm Co) over and above the ICAR (2013) standard in the diets of Sahiwal bull can improve the qualitative and quantitative attributes of semen.

Keywords: Sahiwal,Bull, Trace Mineral, Semen quality

INTRODUCTION

In India, only 25% of the breedable bovine population is under artificial insemination (AI) coverage with a conception rate of about 35%; while rest 75% are covered through natural service either by scrub bulls or with bulls of lower genetic potential (DAHDF, 2018). This is leading to deterioration in the production performance and productivity of most of the important indigenous breeds including Sahiwal. One approach is to provide better nutrition in terms of optimum quantity of trace element in the ration of these animals in addition to energy and protein balance of feed.The current ICAR(2013) guidelines do not make adjustments in mineral requirements for cattle based on growth potential, levels of productivity, physiological status, stress levels, breed, or sex.

Trace minerals are involved in several biological reactions and processes either as a component of metalloenzyme and/or cofactors of various enzymes. Among the trace minerals, supplementation of Zn, Cu, Mn and Co should be considered on account of their deficiency in Indian soil and major influences on male reproduction. Zinc has been reported to influence the process of spermatogenesis, controls sperm motility, stabilizes sperm membrane, preserves the ability of sperm nuclear chromatin to undergo de-condensation and modulates sperm functions (Roy *et al.,* 2013). Copper is involved in spermatozoa motility (Krzyzosiak *et al.,* 2000). Manganese is essential for normal bone growth, onset of puberty, reproductive efficiency of cows and energy metabolism. Mammalian spermatozoal 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 attack which results in oxidative stress (Sikka, 1996). Superoxide dismutase plays a major role in the protection of spermatozoa from this oxidative damage and Zn, Cu and Mn are component of superoxide dismutase. The need of cobalt for thymine synthesis, which is required for DNA

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synthesis, explains the biological role of cobalt for cell division, growth and reproduction.Rumen synthesis of vitamin $B_{12}$is vital for optimal reproductive fertility in cows (Suttle, 2010) but no data have been reported on the impact of cobalt deficiency on bull fertility.

Apart from the inherent traits, season is one major factor which influences the reproductive performance of these animals and it exerts its effect through macro and micro climatic factors like temperature, humidity, rainfall and photo-period (Mandal *et al.,* 2000).Bhakat *et al.* (2014) observed that the hot-dry or summer season adversely affect the various biophysical characteristics of semen in Murrah buffalo bulls. Winter was the most favorable season for good quality semen production and the rainy season might be considered as the intermediate between the two extremes. Rowe *et al.* (2014) observed improvement in motile sperm, progressive sperm and motile sperm with rapid motility for Angus and Balancer (Gelbvieh × Angus) bulls supplemented with organic as compared with inorganic trace mineral (Zn, Cu, Co, Mn, Se and I). However, no literature is available on the effects of dietary supplementation of trace minerals and season on semen production performance of Sahiwal bulls. Hence, this experiment was conducted to evaluate the effect of supplemental trace minerals on semen production performance of Sahiwal bulls.

MATERIALS AND METHODS

The experimental design and plan of the present study was duly approved by the Institution Animal Ethics Committee of ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana.Twelve mature semen donating Sahiwal bulls (average age 6.0-6.5 years) were selected from Artificial Breeding Research Centre (ABRC) of ICAR-National Dairy Research Institute, Karnal. Three groups (replicates of 4 animals in each group) were formed based on semen volume and concentration. Proper deworming, vaccination and other management practices were followed as per Institute schedule for all the animals.

Animals were fed individually a basal diet, consisting of roughages (berseem and wheat straw) and concentrate (maize-28%, bajra-5%, groundnut cake-10%, soybean meal-15%, mustard cake-13%, wheat bran-15%, rice polish-10%, mineral mixture-2%, common salt-1%) in the ratio of 80:20 (DM basis) to meet their nutrient requirements as per ICAR (2013). Treatments consisted of: 1) T_1 (basal diet with 40 ppm Zn, 10 ppm Cu, 15 ppm Mn and 0.11 ppm Co) served as a control; 2) T_2 (basal diet with 1.5 times higher Zn, Cu, Mn and Co *i.e.* extra 60 ppm Zn, 15 ppm Cu, 22.5 ppm Mn and 0.165 ppm Co); and 3) T_3 (basal diet with 2 times higher Zn, Cu, Mn and Co *i.e.* 80 ppm Zn, 20 ppm Cu, 30 ppm Mn and 0.22 ppm Co). The inorganic mineral supplements ($ZnSO_4$, $CuSO_4$, $MnSO_4$ and $CoSO_4$) were provided through concentrate mixture in the form of premixes. Feed offered and residue left were measured accurately to determine dry matter intake (DMI) of the animals. Average DMI was calculated at fortnightly interval.

A digestion trial of seven day collection period was conducted during days 90-96 of experimental feeding to assess the digestibility of nutrients. Body weight of animals were recorded before and after digestion trial for two consecutive days prior to offering feed and water. Daily record of feed offered, feed consumed, feed refusal and faeces voided by individual animal in control (T_1) and treatment (T_2 and T_3) groups was maintained during this period. Fresh drinking water was provided free of choice. The composition [DM, OM, CP, EE and total ash] of feeds, residues and faeces samples were determined as per AOAC (2005). The fractions of cell wall constituents (NDF and ADF) were also analysed (Van Soest *et al.,* 1991).

During 120 days of experimental period, blood samples were collected from all the animals by jugular vein puncture in heparinized vaccutainer at 0, 60 and 120 day, mixed well by rotating tubes between palms to ensure proper mixing of blood and anticoagulant and brought to the laboratory after placing in ice box. Then the samples were centrifuged at 3000 rpm for 15 minutes to separate the plasma. SOD activity was measured in RBC hemolysate as per Madesh and Balasubramanian (1998) and catalase activity was assayed by the method of Aebi (1984). For the

estimation of seminal alkaline phosphatase (ALP) activity, LPO activity, total antioxidant capacity and minerals (Mn, Cr, Co, Zn and Cu), semen samples were centrifuged at 5000 rpm at 5ºC for 15 min to and stored at -20ºC. Minerals (Zn, Cu, Mn, Co, Ca and Fe and Ca) were estimated in an air-acetylene flame on an atomic absorption spectrophotometer (Hitachi-5000 series) at a wave length of 213.9, 324.8, 279.6, 240.7, 422.7 and 248.3, respectively, and hollow cathode lamp was used as the light source. Phosphorus was measured spectrophotometrically in the ultraviolet region (340nm) using kit supplied by Avecon Healthcare Pvt Ltd., Ambala.

Sexual behavioural features were recorded at the time of semen collection using CCTV camera video recording. Live dummy were used for semen collection of Sahiwal bulls. Different dummy bulls were used on different days to minimize sexual satiation of bull from same dummy, to provide uniform stimulus pressure and randomize dummy effects. The sexual behaviour scoring was done as described by Anzar *et al.* (1993). Sexual behaviour score was divided into two parts; *libido* score and mating ability score. *Libido* was scored on the basis of reaction time (in seconds), sexual aggressiveness and tactile stimulation. Reaction time (RT), dismounting time (DT) and total time taken in mounts (TTTM) was determined using methodology described by Elrabie *et al.* (2008). The score (%) *libido* of each ejaculate, mating ability and sexual behaviour was computed as described by Singh *et al.* (2015).

Semen samples were collected from all the animals twice a week till 120 day of the trial. Ejaculate volume (ml) was recorded to the nearest of 0.1 ml in graduated glass tube. The concentration of sperm (million/ml) in the fresh semen was determined using haemocytometer as per method described by Salisbury *et al.* (1985). Semen pH was noted immediately after collection of the semen using a digital pH meter (Century, India). Mass motility of spermatozoa was graded on a 0-5 scale, based on the appearance of waves and swirls created by sperm movement when visualized under low power microscopic magnification (10X) as per methods described by Salisbury *et al.*(1985). Sperm livability percentage was determined using Eosin-Nigrosin stain. Percent intact acrosome was assessed by staining the semen smears with Giemsa stain. The percentage of HOST positive sperm was observed by incubating semen with a hypo-osmotic solution at 37°C for 60 min and the swelling of the sperm tail was examined under high power microscopic magnification [40X] (Jeyendran *et al.,* 1984).

Each ejaculate was further diluted with pre-warmed Biox-cell extender (IMV technologies-France) to the desired semen concentration per milliliter. The extender was composed of carbohydrates, mineral salts, buffer, antioxidants, glycerine, antibiotics (Gentamycin, Tylosin, Lincomycin and Spectinomycin), phospholipids and ultra-pure water. All extended semen samples were examined for individual motility at 37°C with the aid of a television monitor connected to a microscope. Semen with greater than 50% motile sperm was used for further processing. The diluted semen was cooled from 37ºC to 5°C in a cold cabinet for 2 h, semen was then packed into 0.25 ml polyvinyl French straw (0.25 ml; IMV, L'agile, France) by filling and sealing machine (IMV, Cedex, France). Straws were placed on trays for at least 4 hours at (5°C) for further equilibration. The straws were then plunged into liquid nitrogen (-196°C) and packaged in plastic goblets for storage in the liquid nitrogen container.Thawing of frozen semen was carried out in a water bath at 37ºC for 45 seconds. Parameters for evaluation tests were performed at the time of 24 hour for frozen semen. Procedure for estimation of qualitative parameters of frozen semen (post thaw motility, livability, abnormal sperm, HOST and acrosome integrity) and ALP activity is same as described above for fresh semen.

Data were analyzed using the general linear models (GLM) procedure of SPSS 16.0 computer package. The following model was used:

$$Yij=\mu + di + eij$$

Where, Yij is the observation

μis the general mean

di is the effect of i^{th} treatment

eij is the random error.

Tukey's test was used to observe statistical significance between treatment groups. Significant differences were accepted if P≤0.05.

RESULTS AND DISCUSSION

Chemical composition of the feed ingredients fed to the experimental animals has been presented in Table 1. The values are within normal range and comparable with values reported in literature. DMI was around 9.5 kg/d and 1.7 kg/100kg BW during the experimental period. The average body weights were 553.50±3.57, 538.50±6.59 and 545.06±7.19(kg) in T_1, T_2, and T_3, respectively (Table 2). Digestibility of nutrients (DM, OM, CP, EE, NDF and ADF) was similar (P>0.05) in all the groups (Table 2). Supplementation of trace minerals at higher levels had no effect on DMI, which is in agreement with the report of Shinde *et al.*(2012), who found that DM intake was not affected due to Cu and Zn supplementation in Malpura rams while comparing organic and inorganic sources. Majumdar (2017) and Tarun (2018) also did not find any difference in DMI after trace minerals supplementation. Several earlier studies in ruminants suggested no improvement in nutrient digestibility due to supplementation of trace minerals (Shinde *et al.,* 2012; Majumdar, 2017; Tarun 2018). Garg *et al.* (2008) also reported similar observation except improvement in digestibility of ADF and cellulose when supplemented 20 mg chelated Zn/kg DM in rams suggesting a positive role of Zn in fibre digestion. Contrary to these results, Mondal *et al.* (2008) observed improved (P<0.05) digestibility of DM,CP,CF, ash, EE, AIA, and NFE in all mineral (Cu, Fe, Zn and Mn) supplemented groups. Zn, Cu, Mn and Co act as a component of several metalloenzymes and as a cofactor for numerous other enzymes; thus influencing normal metabolism. However, trace mineral levels in the ration of control group was optimum as per ICAR recommendation, therefore, supplementation above that level did not influence the nutrient intake and digestibility. Change in body weight was not observed as these were mature animals.

Data pertaining to blood and seminal plasma enzymes and mineral profile in different groups (Table 3) showed that supplementation of trace minerals at 2 X than ICAR recommendation resulted in significant improvement in blood and seminal plasma mineral levels except for Co. Concentration of other minerals (Fe, Ca and P) was within biological range depicting no negative effect of higher levels of supplementation on these minerals. Results revealed higher values (P<0.05) of ALP activity (U/L) in blood and seminal plasma in T_3 group against T_1group; however, T_1 and T_2 groups had similar values. Antioxidant status (SOD, catalase and

Table 1. Chemical composition of experimental diet (% DM)

Parameter	Concentrate	Wheat straw	Berseem fodder
DM	89.81	91.06	15.17
OM	90.38	89.34	87.98
CP	19.72	3.15	16.71
EE	4.49	0.78	2.48
TA	9.63	10.67	12.02
NDF	27.04	72.24	46.50
ADF	14.50	57.07	26.92
Zn (ppm)	32.15	19.92	35.24
Cu (ppm)	11.24	4.32	14.72
Mn (ppm)	40.25	42.76	54.15
Co (ppm)	0.95	0.27	0.63
Fe (ppm)	570.15	543.28	824.54
Ca (%)	1.32	0.19	2.1

TAC activity) was improved (P<0.05) in $T_{3;}$ group while, LPO activity was lower (P<0.05) in T_3 group in blood and seminal plasma. Higher concentration of Zn has negative effect on absorption of other minerals, mainly Cu and Fe. However, in the present study, concentration of other minerals (Fe, Ca and P) was within normal biological range depicting no negative effect of higher levels of supplementation on these minerals. Similarly, most of the researchers found no significant effect on serum Cu and Fe levels on supplementing zinc at 20 ppm (Zn methionine or $ZnSO_4$) in lambs (Garg *et al.,* 2008) and 35 ppm ($ZnSO_4$ or Zn propionate) in crossbred calves (Mandal *et al.,*2008). Garg *et al.* (2008) also did not notice any change in serum Mn content in relation to Zn content in the ration (20 ppm either as $ZnSO_4$ or Zn methionine). Garg *et al.* (2008) in lambs, Shinde *et al.* (2012) in Malpura lambs, Aliarabi *et al.* (2015) in lambs, Ramulu *et al.* (2015) in Murrah calves and Majumdar (2017) in Murrah bulls reported significant increase in blood plasma Zn levels after Zn supplementation. While, Cheng *et al.* (2011) in lambs reported significantly higher blood plasma Cu concentration after Cu supplementation. However, Mandal *et al.* (2008) in crossbred calves and Rowe *et al.* (2014) in Angus and Balancer bulls did not report any increase in blood plasma concentration of Zn and Cu, respectively, after supplementing trace minerals above recommended level. Improvement of seminal plasma mineral profile was also reported by Majumdar (2017) and Tarun (2018). ALP is a homodimeric enzyme whose active site contains three metal-binding sites (M_1, M_2 and M_3). The M_1 and M_2 sites are occupied by Zn ions while M_3 site is occupied by one Mg^{2+} ion (Stec *et al.,* 2000). Improved blood plasma ALP activity was also observed in lambs (Aliarabi *et al.,* 2015) in Murrah buffalo calves (Ramulu *et al.,* 2015), and in Murrah heifers (Nagalakshmi *et al.,* 2016) while comparing low doses of organic and higher inorganic Zn supplementation. A highly significant (P<0.01) increase in seminal plasma alkaline phosphatase activity was observed in Zn supplemented groups as compared to the control (Kumar *et al.,* 2006). Better antioxidant response and lowered lipid peroxidation as observed in the present study is in accordance with Nagalakshmi *et al.* (2016), they reported that Zn proteinate lowered lipid peroxidation and increased erythrocyte catalase, GPx and SOD activity in Murrah buffalo heifers. Plasma MDA concentrations were not affected (P>0.05) on day 30, but were decreased (*P*<0.001) on day 60 by Cu supplementation. MDA concentrations were decreased in liver tissues (P<0.05), but were not affected in muscle tissues supplemented with Cu compared with the controls in lambs (Cheng *et al.,* 2011). Aliarabi and Chabbra (2006) reported that

Table 2. Effect of dietary supplementation of trace minerals on intake and digestibility of nutrients of Sahiwal bulls

Parameter	Group		
	T_1	T_2	T_3
DMI (kg/d)	9.70±0.10	9.37±0.11	9.62±0.11
DMI (kg/100 Kg BW)	1.75±0.01	1.74±0.01	1.77±0.01
BW (kg)	553.50±3.57	538.50±6.59	545.06±7.19
Digestibility (%)			
DM	61.06±0.63	60.87±0.77	61.32±1.09
OM	63.10±0.67	62.32±1.41	63.17±1.26
CP	61.96±0.65	61.81±1.09	62.02±0.99
EE	71.22±0.44	72.73±1.00	72.32±0.52
NDF	50.60±0.66	51.34±0.83	51.08±0.58
ADF	40.72±0.61	40.15±1.03	41.70±0.85

40ppm chelated Zn supplementation in crossbred calves increased (P<0.05) SOD activity. Senthil Kumar *et al.* (2008) reported higher (P<0.05) hepatic SOD activity in animals supplemented with 14 mg/kg of $CuSO_4$ in lambs using Cu supplementation at 7 or14 mg/kg DM in the form of sulphate or proteinate.

No difference (P>0.05) in any of the sexual behavioral parameters of Sahiwal bulls receiving graded levels of study trace minerals was observed throughout the trial (Table 4). Animals showed moderate libido based on libido score. Mating ability score was around 80%, while sexual behaviour score was around 70% among the groups. Although there was a trend towards improving the behaviour on addition of these minerals in bulls ration. Zn seems to have regulatory role inthe circulating level of dihydrotestosterone by modulating the 5α-reductase activity (the enzyme which converts testosterone to dihydrotestosterone), which is located in the microsomal and nuclear fraction of the prostate.There is also some evidence that Zn plays role in normal functioning of the hypothalamo-pituitary-gonadal axis. Mn is needed for cholesterol synthesis, which ultimately is required for synthesis of the steroids, estrogen, progesterone and testosterone. But, in our experiment,

Table 3. Blood and seminal plasma biochemicals in different groups

Parameter	Group		
	T_1	T_2	T_3
Blood ALP activity (U/L)	72.71^{b}±0.93	73.48^{b}±1.16	92.68^{a}±4.66
LPO (nmol MDA/mL) in blood	4.42^{a}±0.17	3.96^{ab}±0.19	3.76^{b}±0.19
Blood SOD (U/mg of Hb)	23.52^{b}±0.45	24.56^{ab}±0.61	26.05^{a}±0.69
Plasma catalase activity (µmol of H_2O_2 consumed/min/mg Hb)	1.17^{b}±0.02	1.18^{b}±0.04	1.35^{a}±0.05
Plasma TAC (mM/L)	1.31^{b}±0.02	1.34^{ab}±0.02	1.39^{a}±0.03
ALP (U/100 mL) in fresh semen	171.74^{b}±1.49	172.73^{b}±1.38	179.22^{a}±2.01
ALP (U/100 mL) in frozen semen	330.40^{b}±1.80	330.52^{b}±1.76	336.35^{a}±2.13
LPO (nmol MDA/mL of seminal plasma) in fresh semen	8.49^{a}±0.16	8.21^{ab}±0.17	7.82^{b}±0.19
Blood plasma			
Zn (ppm)	1.06^{b}±0.07	1.07^{b}±0.07	1.37^{a}±0.10
Cu (ppm)	0.92^{b}±0.06	0.98^{b}±0.06	1.17^{a}±0.10
Mn(ppm)	0.16^{b}±0.01	0.19^{b}±0.02	0.23^{a}±0.02
Co (ppm)	0.43±0.04	0.44±0.04	0.47±0.05
Fe (ppm)	2.04±0.12	1.98±0.19	2.02±0.13
Ca (%)	9.88±0.42	9.98±0.40	10.01±0.38
P (%)	4.94±0.36	4.68±0.34	4.66±0.26
Seminal minerals			
Zn (ppm)	29.67^{b}±0.74	30.10^{b}±0.77	33.51^{a}±0.95
Cu (ppm)	2.08^{b}±0.02	2.09^{b}±0.03	2.14^{a}±0.02
Mn(ppm)	1.56^{b}±0.03	1.58^{b}±0.05	1.73^{a}±0.04
Co (ppm)	5.59±0.53	5.67±0.58	5.81±0.41
Fe (ppm)	5.72±0.14	5.77±0.18	5.73±0.19
Ca (%)	13.39±0.70	15.46±0.62	13.73±0.78
P (%)	33.66±0.73	32.42±0.93	35.43±0.84

sexual behaviour was not improved by trace mineral supplementation as in mature animal it is a complex phenomenon controlled by many factors *viz.,* level of testosterone, external environment (high temperature, slippery floor, time of day, noise, stress), prior experience *etc.* masking the effect of nutrition. Similar to our findings, Tarun (2018) didn't observe any change in sexual behaviour of mature Sahiwal bulls supplemented with Mn. Co and Cr at higher levels.

Semen quantitative parameters *viz.*, semen volume, sperm concentration and sperm output per ejaculate (million)were higher ($P<0.05$) in T_3 group (Table 4). Semen qualitative parameters like live sperm count, acrosome integrity and HOST positive sperm count were improved in T_3 group in fresh and frozen semen except pH, mass motility, post thaw motility. Abnormal sperm count in fresh semen was reduced ($P<0.05$) in T_3 group but in frozen semen the effect was non-significant. Semen volume mainly consists of secretion of the testes, epididymis and accessory sex glands, especially prostate gland. Zn has been reported to stimulate growth and development of primary, secondary and accessory sex organs as evidenced by atrophy of these organs in rams, when fed a Zn deficient diet. So, enhanced semen volume by Zn supplementation may be attributed to increased secretory activity of prostatic cells, since 35-40% semen volume is contributed by the prostate gland. Similarly, increased semen volume due to Zn supplementation has been reported by Kumar *et al.* (2006) in crossbred bulls, Shinde *et al.* (2012) in Malpura rams, Sabhapati *et al.* (2016) in crossbred bulls and Majumder (2017) in Murrah bulls. While, Roy (2006) reported no effect of supplementation of trace minerals on semen volume of rams and bulls. The results were in accordance with the earlier findings of Kumar *et al.* (2006) in crossbred bulls, Roy (2006) in Murrah and crossbred bulls, and Majumdar (2017) in Murrah bulls.

Improved livability, acrosome integrity and HOST count of sperm in fresh as well as in frozen semen observed in the present study may be due to the membrane stabilizing action of Zn, anti-oxidant

Table 4. Effect of dietary supplementation of trace minerals on different fresh and frozen semen parameters

Parameter	Group		
	T_1	T_2	T_3
Fresh semen			
Volume (mL)	$3.54^{b}\pm0.10$	$3.60^{b}\pm0.11$	$4.01^{a}\pm0.13$
Concentration (million/mL)	$1170.28^{b}\pm12.79$	$1185.53^{b}\pm14.01$	$1234.11^{a}\pm17.51$
Sperm output per ejaculate (million)	$4123.68^{b}\pm97.99$	$4278.01^{b}\pm149.55$	$4980.28^{a}\pm201.43$
pH	6.62±0.01	6.58±0.02	6.60±0.02
Mass activity (0 to 5 scale)	2.33±0.07	2.36±0.08	2.46±0.08
Live sperm (%)	$76.17^{b}\pm0.91$	$76.53^{b}\pm0.83$	$79.47^{a}\pm0.81$
Abnormal sperm (%)	$9.58^{a}\pm0.36$	$8.75^{ab}\pm0.22$	$8.44^{b}\pm0.24$
Acrosome integrity (%)	$87.17^{b}\pm0.54$	$88.36^{ab}\pm0.51$	$90.03^{a}\pm0.60$
HOST (%)	$56.86^{b}\pm0.59$	$57.92^{ab}\pm0.61$	$59.89^{a}\pm0.65$
Frozen semen			
Post thaw motility (%)	52.75±0.52	53.25±0.58	54.42±0.69
Live sperm (%)	$57.17^{B}\pm0.72$	$57.92^{AB}\pm0.75$	$60.08^{A}\pm0.81$
Abnormal sperm (%)	20.67±0.45	19.92±0.48	19.33±0.61
Acrosome integrity (%)	$62.33^{b}\pm0.43$	$62.75^{b}\pm0.73$	$64.75^{a}\pm0.82$
HOST (%)	$44.08^{b}\pm0.57$	$44.83^{b}\pm0.59$	$46.08^{a}\pm0.77$

properties of supplemented minerals (Zn, Cu and Mn). Zn stabilizes membranes by reacting with sulfhydryl groups of membrane proteins to form stable mercaptides and by inhibition of lipid preoccupation through the inhibition of phospholipases (Bettger and O'Dell, 1981). It reversibly binds to highly unsaturated fatty acid phospholipid side chains such as arachidonic acid. By virtue of which, it prevents leakage of enzymes, proteins and other vital components of sperm, thus extending the functional life of sperm. Seminal plasma contains three main enzymatic antioxidants: superoxide dismutase (SOD), catalase, and glutathione peroxidase/ glutathione reductase (GPx/GRD). There are two forms of superoxide dismutase (SOD), the cytosolic Cu/ZnSOD and the mitochondrial Mn/FeSOD. Further, Zn has been found to stabilize various acrosomal enzymes like acrosin, acid phosphatase and phospholipase, which may account for improved intact acrosome percentage. *In vitro* study with water buffaloes concluded that addition of $CuSO_4$ (0.032 mg/l) to semen extenders led to a significant increase in sperm progressive motility, viability, membrane integrity and total antioxidant capacity during freezing processes and reduce the percentage of sperm with damagedDNA after semen freeze-thawing, which in turn, led to improve semen fertility. However, addition of higher Cu concentrations (0.064 mg/l) was detrimental to spermatozoa (Tabassomi and Alavi-Shoushtari, 2013). Bansal and Bilaspuri (2008) suggested that supplementation of Mn^{2+} to the bull sperm enhances the acrosome reaction by decreasing the oxidative stress. Improvement of these parameters after trace minerals supplementation was also reported by Kumar *et al.* (2006) in crossbred bulls, Roy (2006) in Murrah bulls, Sabhapati *et al.* (2016) in crossbred bulls, Majumdar (2017) in Murrah bulls and Tarun (2018) in Sahiwal bulls. But, Roy (2006) and Majumdar (2017) reported no significant improvement in live sperm percentage in trace mineral supplemented groups.

Reduction of sperm abnormality could be attributed to improved spermatogenesis due to Zn supplementation which may result in sperm maturation, motility and fertilizing capacity. Also Zn is required for sperm maturation during the final stage of spermatogenesis (Rodriguez *et al.,* 1985). Si *et al.* (1990) reported that Zn supplementation improved sperm morphology of bull sperm. Further, antioxidant property of supplemented minerals (Zn, Cu and Mn) might be the reason for reduction in total abnormality of semen.

CONCLUSIONS

Dietary supplementation of Zn, Cu, Mn and Co upto 80 ppm, 20 ppm, 30 ppm and 0.22 ppm did not alter BW, DMI, and digestibility of nutrients (DM, CP, EE, NDF and ADF). Semen production performances *viz.,* volume, sperm concentration, live sperm count, intact acrosome and HOST positive sperm count in fresh and frozen semen were improved in the above mentioned groups. Blood and seminal biochemical parameters and mineral levels (Zn, Cu and Mn) were improved due to supplementation. Based on these results, it may be concluded that Zn, Cu, Mn and Co upto 80 ppm, 20 ppm, 30 ppm and 0.22 ppm supplementation will be beneficial in improving the quality of bull semen.

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REFERENCES

Aebi, H. 1984. Catalase *in vitro*. In: *Methods in Enzymology* (L. Packer; ed.). Academic Press, New York. USA. pp. 121-26.

Aliarabi and Chhabra. 2006. Effect of inorganic and chelated zinc supplementation on the performance of crossbred calves. *Indian J. Anim. Nutr.* 23: 141-145.

Aliarabi, H.F., Amir,T., Mohammad M.,Zamani, P., Bahari, A., Farahavar, A. and Dezfoulian and Amir, H. 2015. Effect of Zinc source on hematological, metabolic parameters and mineral balance in lambs. *Biol. Trace Elem. Res.* 168: 82-90.

Anzar, M., Ahmad, M., Nazir, M., Ahmad, N. and Shah, I.H. 1993. Selection of buffalo bulls: Sexual behavior and its relationship to semen production and fertility. *Theriogenol.* 40: 1187-1198.

AOAC. 2005. *Official Methods of Analysis* 18thedn. Association of Official Analytical Chemists. Maryland, USA.

Bansal, A.K. and Bilaspuri, G.S.2008. Effect of manganese on bovine sperm motility, viability, and lipid peroxidation

in vitro. *Anim. Reprod.* 5: 90-96.

Bettger, W.J. and O'Dell, B. 1981. A critical physiological role of zinc in the structure and function of biomembrane. *Life Sci*. 8: 1425-1438.

Benzie, E.F.I. and Strain, J.J. 1999. Ferric reducing /antioxidant power assay: Direct measurement of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant and ascorbic acid concentration. *Methods Enzymol.* 299: 15-27.

Bhakat, M., Mohanty, T.K., Gupta, A.K. and Abdullah, M. 2014. Effect of season on semen quality of crossbred (Karan Fries) bulls.*Adv. Anim.Vet. Sci.* 2: 632-637.

Cheng, J., Ma, H., Fan, C., Zhang, Z., Jia, Z., Zhu, X. and Wang L. 2011. Effects of Different Copper Sources and Levels on Plasma Superoxide Dismutase, Lipid Peroxidation, and Copper Status of Lambs. *Biol. Trace Elem. Res.*144: 570-579.

DADHF. *Annual Report*.Department of Animal Husbandry, Daring and Fisheries. Ministry of Agriculture, Govt. of India. 2018.

Elrabie, K.A., Raina, V.S., Gupta, A.K. and Mohanty T.K. 2009. Effect of semen collection floor on sexual behaviour and semen quality of Sahiwal bulls. *Indian J. Dairy Sci.* 62: 114-118.

Garg, A.K., Mudgal, Vishal and Dass, R.S. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Anim. Feed Sci. Technol.* 144: 82-96.

ICAR. 2013. *Nutrient Requirements of Cattle and Buffalo*, 1st edition, Indian Council of Agricultural Research, New Delhi, India.

Jeyendran, R.S, Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J.Reprod. Fertil*. 70: 219-228.

Krzyzosiak, J., McMillan, G., Molan, P. and Vishwanath, R. 2000. Protein tyrosine phosphorylation during prolonged in vitro incubation of ejaculated bovine spermatozoa is regulated by the oxidative state of the medium. *Biol. Reprod.* 62: 1615-23.

Kumar, N., Verma, R.P, Singh, L.P., Varshney, V.P. and Dass, R.S. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bosindicus* × *Bostaurus)* bulls. *Reprod. Nutr. Dev.* 46: 663-675.

Madesh, M. and Balasubramanian, K.A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian. J. Biochem. Biophys.* 35: 184-188.

Majumder, A. 2017. *Role of Dietary Copper and Zinc Supplementation on Reproductive Performance of Murrah Bulls.* M.V.Sc. Thesis, National Dairy Research Institute, Karnal, Haryana.

Mandal, D.K., Nagpaul, P.K. and Gupta, A.K. 2000. Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. *Indian J. Dairy Sci*. 53: 278-283.

Mandal, G.P., Dass, R.S.,Garg, A.K.,Varshney, V.P. and Mondal, A.B. 2008. Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. *J. Anim. Feed Sci*. 17: 147-156.

Mondal, S., Paul, S.K., Bairagi, B., Pakhira, M.C and Biswas, P. 2008. Comparative studies of reducing level of organic with inorganic trace minerals supplementation on the performance, nutrient digestibility and mineral balance in cross-bred male calves. *Livest. Res. Rural Develop.* 20: 7.

Nagalakshmi, D., Rao, K.S., Kumari, G.A., Sridhar, K. and Satyanarayana, M. 2016. Comparative evaluation of organic zinc supplementation as proteinate with inorganic zinc in buffalo heifers on health and immunity. *Indian J. Anim. Sci.* 86: 322-328.

Ramulu, S.P., Nagalakshmi, D. and Kishan Kumar, M.2015. Effect of zinc supplementation on haematology and serum biochemical constituents in Murrah buffalo calves. *Indian J. Anim. Res.* 49: 482-486.

Rodriguez, H., Ohanian, C. and Bustos-Obregon, E. 1985. Nuclear chromatin decondensation of spermatozoa *in vitro*: a method for evaluating the fertilizing ability of ovine semen. *Int. J. Androl*. 8: 147-158.

Rowe, M.P., Powell, J.G., Hegley, E.B., Lester, T.D. and Rorie, R.W. 2014. Effect of supplemental trace-mineral source on bull semen quality. *Prof. Anim. Sci*. 30: 68-73.

Roy, B. 2006. *Influence of Zinc Supplementation on Semen Quality and Sexual Behaviour of Crossbred and Murrah Buffalo Bulls*. Ph.D. Thesis, National Dairy Research Institute, Karnal, Haryana.

Roy,B., Baghel, R.P.S.,Mohanty T.K. and Mondal, G. 2013.Zinc and male reproduction in domestic animals: A review. *Indian J. Anim. Nutr*. 30: 339-350.

Sabhapati, M., Raina, V.S., Bhakat, M., Mohanty, T.K., Shivahre, P.R., Mondal, G. and Gupta, A.K. 2016. Improvement of sexual behavior and semen quality by therapeutic approach and zinc supplementation on Karan Fries. *Indian J. Anim. Sci.* 86: 655-58.

Salisbury, G.W., Van Demark, N.L., Lodge, J.R., 1985. *Physiology of Reproduction and Artificial Insemination of Cattle*. 2nd ed. CBS Publ. and Dist., New Delhi, India.

Senthil Kumar, P., Nagalakshmi, D. and Ramana Reddy, Y. 2008. Effect of different level and source of copper supplementation on immune response and copper dependent enzyme activity in lambs. *Trop. Anim. Health Prod.* 41: 645-653.

Shinde, A.K., Sankhayan, S.K., Kumar, D. and Regar, R.K. 2012. Effects of supplementation of Cu and Zn on nutrient intake, utilization, blood profile, wool yield and semen quality of Malpura lambs. *Indian J. Small Rumin.* 18: 191-197.

Si, Y.M., Zhang, Z.C., An, M., Lu, X.L., Lu, A. and Wang, L. 1990. Effect of zinc supplementation of the feed on quality of frozen semen from Holstein bulls. *Chinese J. Anim.Sci*. 26: 9-12.

Sikka, S.C. 1996. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front. Biosci.* 1: 78-86.

Singh, S., Bhakat. M., Mohanty, T.K., Kumar, A., Gupta, A.K., Chakravarty, A.K. and Singh, P. 2015. Sexual behavior and its relationship with semen quality parameters in Sahiwal breeding bulls. *Vet. World.* 8: 745-749.

Stec, B., Holtz, K.M. and Kantrowitz, E.R. 2000. A revised mechanism for the alkaline phosphatase reaction involving three metal ions. *J. Mol. Biol*. 229: 1303-1311.

Suttle, N., 2010. *Mineral Nutrition of Livestock*. 4th Ed. Commonwealth Agricultural Bureaux International, Oxfordshire, UK.

Tabassomi, M. and Alavi-Shoushtari S.M. 2013. Effects of *in vitro* copper sulphate supplementation on the ejaculated sperm characteristics in water buffaloes (*Bubalusbubalis*). *Vet. Res. Forum.* 4: 31-36.

Tarun, A. 2018. *Effect of Dietary Supplementation of Manganese, Chromium and Cobalt on Semen Production Performance of Sahiwal Bulls*. M.V.Sc. Thesis, National Dairy Research Institute, Karnal, Haryana.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

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