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EFFECT OF ORGANIC ZINC SUPPLEMENTATION ON GROWTH PERFORMANCE AND SEXUAL BEHAVIOUR AND SEMEN QUALITY IN GIR BULLS

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Abstract: The objective of present study was to study the effect of zinc supplementation (Zn propionate) on sexual behaviour and semen quality of Gir bulls. Eight Gir bulls of age group (nearly 3.0 to 6.5 years) were supplemented with Zn Propionate at the rate 40 ppm per animal for a period of 60 days to complete one spermatogenic cycle. A total 128 ejaculates (64 before and 64 after supplementation) which includes eight ejaculates from each bull were collected and evaluated for semen quantitative and qualitative characteristics. Dry matter intake (kg/day, kg/100 kg BW, kg/$W^{0.75}$) was at par in post supplementation phase. Experimental bulls showed significant ($p<0.05$) increase in body weight gain. Biometry (Heart girth, body length, height) also showed significant ($p<0.05$) change in post supplementation phase. Scrotal measurements also significantly ($p<0.05$) increased in second phase of experiments. Mean ejaculate volume (ml) was significantly ($p<0.001$) higher semen volume during Zn-supplemented period as compared to the non supplemented period. Similarly, sperm concentration (millions/ml), live sperm (%) and motility (%) were significantly ($p<0.001$) higher during Zn-supplemented period as compared to control. The decrease abnormal sperm % in neat and thawed semen was significant ($p<0.001$) during post supplementation period. The results of sexual behaviour, libido and mating ability revealed a significant ($p<0.01$) improvement in all the bulls supplemented with Zn as compared to the control period. It may be concluded that Zn supplementation in organic form in the diet of Gir bulls improved the qualitative and quantitative attributes of semen.

Keywords: Gir bulls, Sexual behaviuor, Semen quality, Zinc propionate.

INTRODUCTION

Zinc is an essential trace element required for the action of more than 200 metallo enzymes and plays an important role in polymeric organization of macromolecules like DNA and RNA, protein synthesis and cell division. Zinc plays an important role in prostate, epididymal and testicular functions (Ebisch *et al*., 2003). Zinc has been reported to influence the process of spermatogenesis (Wong *et al*., 2002), controls sperm motility (Wroblewski *et al.*, 2003), stabilizes sperm membrane (Kendall *et al*., 2000), preserves the ability of sperm nuclear chromatin to undergo decondensation and modulates sperm functions. Hypozinkemia leads to

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gonad dysfunction, decreased testicular weight, atrophy of seminiferous tubules and complete cessation of spermatogenesis. Zinc is found in high concentration in the male reproductive tract as well as in semen. The mean concentration of Zn in the semen of bulls, rams, stallions and boar has been reported as 83.15, 60.46, 86.20 and 171.74 mg per kg wet weight of tissue, respectively (Massonyi *et al*., 2004). The recommended level of zinc in the diet of cattle is 35–40 ppm and is sufficient for normal body functions, but for enhanced immunity, higher levels of zinc have been found beneficial (NRC, 1989). Similarly, supplementation of zinc as organic/ chelated minerals has been found more beneficial as compared to inorganic sources. Till date, very little work has been done in cattle bulls in relation with zinc supplementation.

Nutrition plays critical role right from birth in enhancing the vigour and quality of semen. Feeds and fodders are the main sources of macro and micro nutrients for livestock. Crop residues, which form the bulk of rations in India, are deficient in most of the mineral elements including Zn (Datt and Chhabra, 2005). However, forty eight percent of Indian soils are deficient in Zn (Arunachalam *et al.*, 2013) which may affect the male reproduction. Zn content in animal feeds varies widely due to various factors viz., soil type, seeds, fertilizers, irrigation and climatic conditions. Oil seed cakes, cereal brans and legume seeds contain higher concentration of Zn, followed by green fodders, cereal grains and cereal straws. Organic and inorganic forms of Zn are metabolized differently in the body after absorption and organic form of Zn is better absorbed and utilized in body compared to inorganic Zn (Galyean *et al.,* 1999). Feeding legume fodders and zinc rich concentrate elevate Zn deficiency in ruminant. Therefore, the present investigation is intended to study the effect of dietary supplementation of Zn in the ration on sexual behaviour and semen quality of Gir bulls.

MATERIALS AND METHOD

2.1. Animals, their feeing and management

A total of 8 Gir bulls under semen collection at Cattle Breeding Farm were selected as experimental animals. A total of 128 ejaculates (64 pre-supplementation and 64 post-supplementation) were collected from 8 adult Gir bulls. Representative sample of feeds and fodders were analyzed (AOAC, 1999) for proximate composition and phosphorus. Calcium content in feeds and fodder samples was estimated by Talapatra *et al*. (1942). Zn content in feeds and fodder was analyzed using wet digestion technique (AOAC, 1999) (Table 1). ICAR (2010) feeding standards were followed for meeting the nutrient requirements of breeding bulls. Following were part of the experimental rations: In treatment one commercial

concentrate mixture (ISI –grade –I) and Cotton seed cake to meet protein requirement. Green fodder: 10 kg maize/ seasonal green fodder available on the farm to meet carotene requirement. Dry fodder: Groundnut haulms (6 kg/animal/day) and mature pasture grass hay offered *ad libitum.* To the basal diet given in $T_{1,}$ Zinc Propionate @ 40 ppm/animal was supplemented for a period of 60 days to complete one spermatogenic cycle.

Each bull was individually weighed before offering feed for three consecutive days during every fortnight during the experimental period. Average of these three recordings was considered as mean body weight for feeding calculations. Once in four weeks at the line of weighing each bull was measured for: body length, height at withers, chest girth. Besides biometry of these animals, scrotal measurements were also recorded twice, i.e. once at the beginning of the experiment and second at the end of second phase of experiment. Scrotal measurements recorded as: scrotal circumference, scrotal length, scrotal width.

2.2. Sexual behaviour score of experimental Gir bulls

Weekly two teasings to each bull with using dummy in trevis were done and sexual behaviour of Gir bulls were recorded. Libido and mating ability were also recorded. Libido was scored on the basis of reaction time (in seconds), sexual aggressiveness and tactile stimulation of teaser until mounting was recorded (Anzar *et al*., 1993). Sexual aggressiveness, behaviour of a bull during approach toward the teaser was assessed visually and the bull was classified as aggressive, active, dull or shy (Amann and Almquist, 1976).

Reaction time and sexual aggressiveness were scored on a 6 and 4 point scale, respectively. Tactile stimulation (TS) consists of sniffing, butting, licking perinea, licking urine, licking penis, protrusion and Flehman's reaction (Anzar *et al*., 1993).

2.3. 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 in the first one month to assess semen characteristics of all bulls at the start of the experiment. In the last 2 months of experimental feeding, 8 ejaculates from each animal were collected to study the effect of Zn supplementation on semen characteristics. All semen samples were evaluated for quantitative and qualitative characteristics.

2.4. Collection of semen and evaluation of quantitative characteristics

Quantitative characteristics of semen included ejaculate volume, sperm concentration. 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 530nM wavelength.

2.4.1. Evaluation of qualitative characteristics of semen

It included the assessment of mass motility, individual motility, live and dead sperm percentage and abnormal sperm percentage. 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×). Extremely rapid waves or swirl motion of sperms were given a 5 numerical scale, comparatively slower waves and swirls were given a 4 numerical scale and likewise 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×). The individual motility was recorded as the percentage of progressive motile sperm. Live sperm percentage was calculated by using Eosin- Nigrosin stain. Similarly, the stain utilized for counting abnormal sperm percentage.

2.5. Statistical analysis

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

RESULTS AND DISCUSSION

3.1. Body weight, biometry and scrotal dimensions of Gir bulls

The mean body weight, biometry and scrotal dimension in experimental Gir bulls are presented in Table 2. Body weight of experimental Gir bulls varied from 370 to 680 kg with an overall mean of 491.25 ± 38.00 and 554.67 ± 35.00 kg, respectively, during prior and post supplementation phases of the experiment. Experimental bulls gained 8.00 % of body weight in the second phase of the experiment, which was significant ($p<0.001$). Adult body weight for adult Gir units was reported to be 440 kg and body weights recorded in the present experiment in Gir bulls was higher than reported by (Anonymous, 2015). Mean heart girth (meters) was 1.82 ± 0.05 and 2.00 ± 0.07, respectively, before and after supplementation period in experimental Gir bulls. Body length of Gir bulls was 1.54 ± 0.14 and 1.67 ± 0.13 meters, respectively before and after supplementation period.

Height at withers was recorded to be 1.29 ± 0.03 and 1.37 ± 0.02 meters, respectively before and after supplementation period in Gir bulls. Significant (($p<0.01$) for Heart Girth , ($p<0.02$) for body length and ($p<0.05$) for Height at Withers), changes in body measurements of experimental bulls is explainable due to growth stage of four of the eight experimental

bulls over a period of 100 days.Biometry of bulls is in consonance with the reports of Anonymous, 2015.

During pre-supplementation phase, length of right testis, length of left testis, circumference of right and left testis and total scrotal circumference respectively were (mm) 116.98 ± 0.14, 114.23 ± 3.66, 72.68 ± 2.00, 74.47 ± 2.64, 114.25 ± 4.84. Respective values during post feeding were (mm) 127.36 ± 0.13, 124.06 ± 3.67, 81.24 ± 1.96, 89.89 ± 3.37, 132.73 ± 4.49. At the end of second phase of the experiment, there is significant ($p<0.001$) increase in all the scrotal measurements, mainly due to growing stage of farm born bulls. Abdel *et al*. (2010) reported significantly ($p<0.05$) higher scrotal circumference in Egyptian buffalo bulls offered Selenium, Vitamin E and Zinc as G5 treatment compared to control group of bulls that received no treatment at all. Scrotal circumference in Egyptian buffalo bulls was almost three times that of Gir bulls. However, complimentary effect of Zn with Selenium and Vitamin E was visible with regard to scrotal circumference. In the present study Zn propionate supplementation find significant ($p<0.02$) effect on scrotal biometry and trend is in concurrence with the observations of Abdel *et al*. (2010).

3.2. Dry matter and nutrients intake

The average dry matter and nutrient intake of experimental Gir bulls are presented in Table 3. Overall mean values for DM intake kg/day in Gir bulls was 7.72 ± 0.70 and 8.32 ± 0.40 kg per day, before and after supplementation of Zinc Propionate, respectively, while percent DM intake kg/100kg BW and DM intake g/kg$W^{0.75}$ basis were 1.92 ± 0.21 and 2.11 ± 0.74 and 86.41 ± 1.20 and 93.14 ± 1.08, respectively. DM intake significantly ($p<0.01$) increased due to Zn propionate supplementation. DM intake in the present study was lower than ICAR (2010) recommended intake before supplementation. However, post supplementation of Zn propionate, percent DM intake and DM intake/100 kg BW, DM Intake/kg $W^{0.75}$, in Gir bulls were at par with ICAR (2010) recommended intakes.

Average values for water intake was 38.54 ± 0.74 Lit./day, 9.62 ± 1.12 Lit./100 kg/BW, 432.51 ± 0.33 ml/kg $W^{0.75}$ and 40.01 ± 0.87 Lit./day, 10.00 ± 1.00 Lit./100 kg BW, 450.27 ± 0.21 ml/kg $W^{0.75}$, respectively, before and after supplementation of Zn propionate. Water intake was not significantly affected due to Zn propionate supplementation.Water requirement varied in cattle weighing 500 kg from 5 to 6.5 Lit./kg DMI and 6.5 to 10 Lit./100 kg BW as the environmental temperature varied from 10^0 C to 35^0C (ICAR, 2010).

Mean intake of CP was 512.10 ± 2.11 g/day, 128.52 ± 0.89 g/ 100 kg BW and 5.85 ± 0.23 g/kg $W^{0.75}$and 523.10 ± 1.88 g/day, 130.78 ± 0.98 kg/100 kg BW, and 6.98 ± 0.20 g/kg

$W^{0.75}$, respectively, before and after supplementation of Zn propionate. The difference in CP intake was significant ($p<0.05$).CP requirement suggested by ICAR (2010) was 515 g/day, 103 g/100kg BW and 4.91 g/kg $W^{0.75}$, respectively. However in the present study CP intake during both the phases of experiment was higher than CP requirement suggested by ICAR (2010). This may be attributed to feeding of groundnut haulms which is a leguminous crop by-product offered as dry forage. Four of the eight bulls were growing and hence the growing phase resulted in higher CP intake from consumption of groundnut haulms.

Mean Zn intake was 33.27 ± 1.05 ppm prior to supplementation and 55.59 ± 0.96 ppm post supplementation period, with significant difference. Cupiz *et al*. (1998) obtained better seminal characteristic with 150 ppm of Zn in HF bulls. However, Wenli and Huiyin (1998) recorded optimum level of Zn supplementation to be 110 mg/kg DM intake in his experiment on HF bulls, which was quite higher than reported in present study. ARC (2000) suggested Zn requirement of 40 ppm/day in cow bulls while NRC (2001) suggested 45 mg/day in buffalo bulls for bull weighing 400 kg BW. Roy (2006) reported no beneficial effect of 40 ppm Zn intake in Murrah buffao bulls. However, (Kumar *et al*., 2007) reported 35 ppm/day Zn propionate supplementation resulted in enhancing sexual behavior in Crossbred bulls. Imam *et al*. (2008) concluded that 35 ppm Zn propionate showed better results in comparison to double dose of inorganic Zn (Zn sulphate @ 70 ppm) supplementation in Murrah buffalo bulls.

3.3. Sexual Behaviour score

Sexual behaviour, libido and mating ability score of Gir bulls are presented in Table 4. All the experimental individual bulls showed improvement in libido score, mating ability score and sexual behaviour score post Zn propionate supplementation period and overall these scores improved significantly ($p<0.01$) indicating that Gir bulls responded to Zn propionate supplementation.

Reaction time (RT) was significantly improved following Zn supplementation (ZnO) to Egyptian buffalo bulls (Osman *et al.,* 2000). Kumar *et al*. (2006) reported that crossbred bulls supplemented with 35 ppm Zn reported significantly higher sexual behavior and reaction time in. Whereas, Roy (2006) did not find significant change of the same following Zn supplementation in HF cross and Murrah bulls. Imam *et al*. (2008) reported that supplementation of Zn propionate @ 35 ppm/day to Murrah bulls significantly improved testosterone level in the serum, which was responsible for the libido. Though testosterone

estimation was not done in present study, enhanced libido score, mating ability score and sexual behavior score is attributed to probably enhanced level of testosterone.

3.4. Semen quantitative and qualitative characteristics of Gir bulls after 2 months of Zn supplementation

Mean ejaculate volume, mass motility and individual motility and past thaw motility in semen of Gir bulls are presented in Table 5. Overall mean values for semen volume was 3.3 ± 0.6 and 4.7 ± 0.4 ml per ejaculate, before and after supplementation, respectively, the difference being significant ($p<0.01$). Among individual bulls Ganesh and Paras recorded significantly [($p<0.02$), ($p<0.05$)] increased semen volume from 2.7 ± 0.4 to 4.3 ± 0.5 and 3.3 ± 0.5 to 4.7 ± 0.4 ml respectively. Twenty five per cent of the experimental bulls responded to Zn propionate supplementation with regard to increasing semen volume. Semen volume also increased significantly ($p<0.05$) in Barbari bucks maintained on additional supplementation of Zn and Selenium for a period of 105 days (Pankajkumar *et al.,* 2014). Shelke and Dhami (2001) investigated quality and freezability of Gir bull semen. They reported that the mean ejaculate volume was 4.84 ± 0.16 ml. They found non-significant difference between ejaculate volume and other seminal characteristics. Kumar *et al.* (2006) supplemented Zn in organic as well as inorganic forms in Crossbred bulls and found significant ($p<0.05$) improvement in semen volume in organic Zn supplemented group compared to control group. Regarding mass motility, it was increased by 11.33 per cent during post supplementation period. Though all the bulls showed improvement in mass motility. 62.5 per cent bull showed significant ($p<0.05$, $p<0.01$) increase in mass motility.

With regard to individual motility per cent all the experimental bulls (100%) responded significantly to individual sperm motility. The mean being 79.14 ± 1.96 and 86.74 ± 1.34, respectively before and after supplementation with an overall increase of 9.8 %. In bulls Vasu, Baldev, Shiv ($p<0.01$), Ganesh, Pratap and Raj ($p<0.05$), Sawan ($p<0.02$) and Paras ($p<0.001$) responded positively with enhanced individual motility per cent post supplementation. Post thaw semen motility in present experiment ($p<0.01$) improved in post supplementation period compared to control.The mean post thaw motility was 55.05 ± 1.4 and 61.0 ± 1.3 % during pre and post supplementation period, respectively.

3.5. Average neat semen attributes in semen of experimental Gir bulls

Mean sperm concentration, per cent live and dead sperm per cent are presented in Table 6. Mean values for sperm concentration was 1306 ± 71.88 and 1447± 71.84 millions/ml respectively, before and after supplementation period. The difference was significant

($p<0.001$). Post supplementation appeared to have increased live sperm per cent significantly ($p<0.001$).

Mean live sperm per cent was 81.33 ± 1.93 and 88.28 ± 1.17, respectively pre and post supplementation of Zn propionate. Except Pratap all the individual Gir bulls responded significantly with regard to live sperm count. Raj and Sawan ($p<0.05$), Ganesh, Paras, Baldev and Shiv ($p<0.01$), Vasu ($p<0.02$) responded significantly to Zn propionate supplementation with regard to live sperm count. The mean sperm abnormalities in neat and thawed semen are presented in Table 7 and 8. Total abnormalities in neat semen of Gir bulls reduced significantly by 27.00 %. The mean total abnormalities were 15.75 ± 0.7 and 10.57 ± 0.8 % pre and post supplementation, respectively. The reduction was significant at $p<0.001$ % in Vasu, Sawan and Shiv, $p<0.02$ % in Ganesh, Pratap, Paras and Raj, $p<0.05$ % in Baldev.

Fertility of bulls is determined by not only concentration but also by progressive motile spermatozoa and the number of progressive motile sperm are directly relation to spermatozoa abnormality in neat semen as well as post thawed semen for successful AI. Abnormal sperm per cent reduced in grazing Rams supplemented with additional Zn (Kendall *et al*., 2000).

Conclusion

It may be concluded that supplementation of Zn in the diet of Gir bulls improved semen quality in terms of quantitative and qualitative characteristics of semen, as compared to the non-supplemented period.

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Table 1: Proximate composition (% on DM basis) of feeds and fodder used in the experiment

Constituent	Concentrate Mixture	Cotton seed cake	Seasonal green	Groundnut haulms	Mature pasture grass hay
Dry matter %	89.20	91.50	35.00	95.00	85.00
Organic matter %	93.75	96.11	94.29	96.43	89.05
C.Protein %	21.50	36.20	10.60	11.50	02.12
C.Fibre %	10.71	19.96	25.50	31.00	28.70
E.Extract %	04.00	10.33	01.66	01.73	01.22
NFE %	57.54	29.62	56.53	52.20	57.01
Ash %	06.25	03.89	05.71	03.57	10.95
Silica %	03.28	02.40	02.78	02.13	08.75
Calcium %	01.19	00.49	01.34	00.60	00.40
Phosphorus %	00.76	00.19	01.02	00.20	00.15
Zinc (ppm)	59.30	39.65	09.34	27.10	03.00

Table 2: Changes in mean body weight, biometry and scrotal dimension in experimental Gir bulls (Overall Mean ± SE)

Measurement			Pre feeding	Post feeding	T value	Significance
1.		Body weight (kg)	491.25 ± 38.00	554.67 ± 35.00	9.39	0.001
2.		Biometry				
	2.1	Heart girth (meter)	1.82 ± 0.05	2.00 ± 0.07	5.38	0.01
	2.2	Body length (meter)	1.54 ± 0.14	1.67 ± 0.13	3.96	0.02
	2.3	Height at wither (meter)	1.29 ± 0.03	1.37 ± 0.02	2.51	0.05
3.		Scrotal dimensions				
	3.1	Length of right testis (mm)	116.98 ± 0.14	127.36 ± 0.13	14.85	0.001
	3.2	Length of left testis (mm)	114.23 ± 3.66	124.06 ± 3.67	8.96	0.001
	3.3	Circumference of right testis (mm)	72.68 ± 2.00	81.24 ± 1.96	13.92	0.001
	3.4	Circumference of left testis (mm)	74.47 ± 2.64	89.89 ± 3.37	13.00	0.001
	3.5	Scrotal circumference (mm)	114.25 ± 4.84	132.73 ± 4.49	8.30	0.001

Table: 3 Average dry matter and nutrient intake of experimental Gir bulls before and after Zn supplementation (Overall Mean ± SE)

Measurements	Pre supplementation	Post supplementation	T value	Significance
1. DM intake kg/day	7.72 ± 0.70	8.32 ± 0.40	5.22	0.01
kg/100kg BW	1.92 ± 0.21	2.11 ± 0.74	5.22	0.01
g/kg $W^{0.75}$ BW	86.41 ± 1.20	93.14 ± 1.08	5.22	0.01
2. Water intake lit./day	38.54 ± 0.74	40.01 ± 0.87	0.57	NS
Lit./100kg BW	9.62 ± 1.12	10.00 ± 1.00	0.57	NS
ml/kg $W^{0.75}$ BW	432.51 ± 0.33	450.27 ± 0.21	0.57	NS
3. Protein intake g/day	512.10 ± 2.11	523.10 ± 1.88	2.69	0.05
g/100kg BW	128.52 ± 0.89	130.78 ± 0.98	2.69	0.05

g/kg $W^{0.75}$ BW	5.85 ± 0.23	6.98 ± 0.20	2.69	0.05
4. Zinc intake ppm	33.27 ± 1.05	55.69 ± 0.96	2.39	0.05

Table 4: Average per cent libido, mating ability and sexual behavior score of experimental Gir bulls supplemented with Zn propionate.

Bull Name	Libido score (%)		Mating ability score (%)		Sexual behavior score (%)	
	Pre supplementation	post supplementation	Pre supplementation	post supplementation	Pre supplementation	post supplementation
Vasu	60.12 ± 4.3	80.24 ± 4.3	55.57 ± 2.8	75.74 ±3.8	57.51 ± 2.8	77.50 ± 3.4
Ganesh	48.51 ± 4.3	62.24 ± 4.2	60.84 ± 2.1	80.98 ± 3.7	54.51 ± 2.7	71.98 ± 2.8
Pratap	40.14 ± 4.2	50.45 ± 4.3	65.12 ± 2.2	75.87 ± 3.6	52.57 ± 1.7	62.50 ± 2.2
Paras	34.55 ± 4.0	50.45 ± 4.0	30.41 ± 4.1	45.47 ± 3.3	32.26 ± 1.4	60.54 ± 2.4
Sawan	60.89 ± 3.3	80.19 ± 3.9	35.23 ± 3.1	75.56 ± 3.0	57.56 ± 2.8	77.50 ± 2.8
Baldev	50.98 ± 3.8	54.89 ± 2.8	60.44 ± 2.8	60.14 ± 2.8	55.78 ± 4.2	57.50 ± 2.8
Shiv	58.57 ± 4.8	64.95 ± 4.0	65.24 ± 2.9	70.12 ± 2.4	61.53 ± 4.5	67.51 ± 2.9
Raj	72.11 ± 2.1	72.22 ± 2.2	80.87 ± 2.2	85.91 ± 4.0	76.11 ± 2.3	75.50 ± 2.4
Overall mean ± SE	53.23 ± 3.8	64.45 ± 3.7	56.71 ± 2.7	71.22 ± 3.3	55.97 ± 2.8	68.81 ± 2.7
T value	4.808		4.203		3.588	
Significance	0.01		0.01		0.01	

Table 5: Mean ejaculate volume, mass motility and individual motility and past thaw motility in semen of Gir bulls.

Characteristics	Bull Name	Treatment			
		Pre supplementation	Post supplementation	T value	Significance
Ejaculate Volume (ml)	Vasu	3.6 ± 0.7	4.4 ± 0.2	0.68	NS
	Ganesh	2.7 ± 0.4	4.3 ± 0.5	3.25	0.02
	Pratap	4.0 ± 0.9	5.1 ± 0.9	1.60	NS
	Paras	3.3 ± 0.5	4.7 ± 0.4	2.87	0.05
	Sawan	3.1 ± 0.6	5.1 ± 1.0	1.79	NS
	Baldev	3.6 ± 0.6	5.1 ± 1.1	1.77	NS
	Shiv	2.7 ± 0.5	4.6 ± 0.1	1.73	NS
	Raj	3.7 ± 0.7	4.3 ± 0.4	0.75	NS
Overall mean ±SE		**3.3 ± 0.6**	**4.7 ± 0.6**	**7.33**	**0.001**
Mass Motility	Vasu	68.30 ± 0.72	73.75 ± 0.82	1.16	NS

(%)	Ganesh	69.20 ± 0.45	78.32± 0.45	2.43	0.05
	Pratap	60.10 ± 0.15	71.24 ± 0.15	2.43	0.05
	Paras	50.70 ± 0.15	57.14 ± 0.15	2.01	NS
	Sawan	67.11 ± 0.08	77.25 ± 0.01	2.67	0.05
	Baldev	71.82 ± 0.45	78.25 ± 0.12	2.39	0.05
	Shiv	68.10 ± 0.15	72.96 ± 0.12	1.16	NS
	Raj	67.72 ± 0.71	77.22 ± 0.41	4.47	0.01
Overall mean ±SE		**65.38 ± 0.35**	**73.26 ± 0.27**	**6.92**	**0.001**
Individual motility (%)	Vasu	81.87 ± 0.91	87.57 ± 1.34	3.81	0.01
	Ganesh	81.25 ± 1.62	85.22 ± 1.98	2.36	0.05
	Pratap	80.62 ± 1.77	85.60 ± 0.68	2.60	0.05
	Paras	77.52 ± 1.33	87.50 ± 1.65	7.48	0.001
	Sawan	80.00 ± 1.67	87.50± 1.35	3.24	0.02
	Baldev	77.50 ± 2.38	85.00 ± 1.85	3.96	0.01
	Shiv	82.50 ± 1.33	89.30 ± 0.65	5.23	0.01
	Raj	71.87 ± 4.71	86.25 ± 1.22	2.67	0.05
Overall mean ±SE		**79.14 ± 1.96**	**86.74 ± 1.34**	**6.48**	**0.001**
Post thaw Motility (%)	Vasu	49.37 ± 1.4	58.75 ± 2.1	4.25	0.01
	Ganesh	56.25 ± 0.8	58.75 ± 1.2	1.3	NS
	Pratap	61.25 ± 0.8	65.62 ± 1.7	2.19	NS
	Paras	55.00 ± 0.0	61.27 ± 1.3	5.22	0.01
	Sawan	55.60 ± 1.7	60.62 ± 0.6	3.05	0.05
	Baldev	58.70 ± 0.8	61.80 ± 1.3	2.37	0.05
	Shiv	55.60 ± 1.1	60.60 ± 1.1	2.62	0.05
	Raj	48.70 ± 4.6	60.60 ± 1.1	2.62	0.05
Overall Mean ±SE		**55.05 ± 1.4**	**61.00 ± 1.3**	**5.25**	**0.01**

Table 6: Average neat semen attributes in semen of experimental Gir bulls

Characteristics	**Bull Name**	**Treatment**			
		Pre supplementation	Post supplementation	T value	Significance
Sperm concentration (Millions/ml)	Vasu	1109 ± 94.98	1211 ± 91.28	0.73	NS
	Ganesh	1284 ± 69.27	1446 ± 83.19	2.23	NS
	Pratap	1289 ± 68.64	1394 ± 62.88	3.03	0.02
	Paras	1072 ± 73.02	1253 ± 78.10	4.95	0.01
	Sawan	1325 ± 38.55	1460 ± 57.58	3.01	0.02
	Baldev	1605 ± 98.80	1745 ± 97.00	3.20	0.02
	Shiv	1394 ± 59.20	1560 ± 54.54	2.05	NS
	Raj	1371 ± 72.64	1511 ± 50.21	2.86	0.05

Overall mean ± SE		**1306 ± 71.88**	**1447 ± 71.84**	**14.4**	**0.001**
Live sperm (%)	Vasu	80.60 ± 1.20	87.30± 1.20	4.20	0.02
	Ganesh	82.50± 1.20	90.70± 1.20	5.38	0.01
	Pratap	79.30± 1.71	82.90± 1.41	1.81	NS
	Paras	82.70 ± 2.21	90.10± 1.21	3.54	0.01
	Sawan	84.40± 0.91	89.40± 1.21	2.90	0.05
	Baldev	85.80± 1.52	92.00± 0.82	3.26	0.01
	Shiv	83.10± 1.88	88.80 ± 0.82	3.26	0.01
	Raj	72.20± 4.81	85.10 ± 1.51	2.39	0.05
Overall mean ± SE		**81.33 ± 1.93**	**88.28 ± 1.17**	**7.01**	**0.001**
Dead sperm (%)	Vasu	19.40 ± 1.20	12.70± 1.20	4.20	0.02
	Ganesh	17.50± 1.20	09.30± 1.20	5.38	0.01
	Pratap	20.70± 1.71	17.10± 1.41	1.81	NS
	Paras	17.30± 2.21	09.90± 1.21	3.54	0.01
	Sawan	15.60±0.91	10.60±1.21	2.90	0.05
	Baldev	14.20± 1.52	08.00± 0.82	3.26	0.01
	Shiv	16.90± 1.88	11.20± 0.82	3.26	0.01
	Raj	27.80± 4.81	14.90± 1.51	2.39	0.05
Overall mean ± SE		**18.67 ± 1.93**	**11.72 ± 1.17**	**7.01**	**0.001**

Table 7: Average semen attributes in thawed semen of experimental Gir bulls

Characteristics	**Bull Name**	**Treatment**			
		Pre supplementation	Post supplementation	T value	Significance
Live sperm (%)	Vasu	58.6 ± 1.5	62.2 ± 1.2	1.72	NS
	Ganesh	55.2 ± 1.9	66.3 ± 1.0	6.29	0.001
	Pratap	56.7 ± 1.7	63.7 ± 1.6	2.95	0.05
	Paras	59.0 ± 2.7	67.0 ± 2.0	2.85	0.05
	Sawan	56.7 ± 1.8	61.5 ± 2.1	2.54	0.05
	Baldev	56.3 ± 2.8	63.9 ± 2.2	3.55	0.01
	Shiv	63.3 ± 1.4	68.2 ± 2.1	2.05	NS
	Raj	55.9 ± 2.1	63.3 ± 1.5	2.75	0.05
Overall mean ±SE		**57.7 ± 1.9**	**64.5 ± 1.7**	**8.16**	**0.001**

Dead sperm (%)	Vasu	41.4 ± 1.5	37.8 ± 1.2	1.72	NS
	Ganesh	44.8 ± 1.9	33.7 ± 1.0	6.29	0.001
	Pratap	43.3 ± 1.7	36.3 ± 1.6	2.95	0.05
	Paras	41.0 ± 2.7	33.0 ± 2.0	2.85	0.05
	Sawan	43.3 ± 1.8	38.5 ± 2.1	2.54	0.05
	Baldev	43.7 ± 2.8	36.1 ± 2.2	3.55	0.01
	Shiv	36.7 ± 1.4	31.8 ± 2.1	2.05	NS
	Raj	44.1 ± 2.1	36.7 ± 1.5	2.75	0.05
Overall mean ±SE		**42.3 ± 1.9**	**35.5 ± 1.7**	**8.16**	**0.001**

Table 8: Average sperm abnormality in neat and thawed semen of experimental Gir bulls

Sperm abnormalities of neat semen					
Characteristics	**Bull**	**Treatment**			
		Pre supplementation	Post supplementation	T value	Significance
Total abnormality (%) (Head, Mid piece, Tail)	Vasu	16.50 ± 0.9	09.99 ± 0.6	6.30	0.001
	Ganesh	16.12 ± 0.8	10.75 ± 1.2	3.86	0.02
	Pratap	18.29 ± 0.8	08.07 ± 1.3	3.16	0.02
	Paras	15.74 ± 0.8	11.55 ± 1.2	3.10	0.02
	Sawan	14.49 ± 0.6	10.37 ± 0.7	5.60	0.001
	Baldev	16.45 ± 0.4	14.42 ± 0.5	2.37	0.05
	Shiv	13.62 ± 0.6	09.82 ± 0.6	6.70	0.001
	Raj	14.85 ± 0.7	09.59 ± 0.5	4.33	0.02
Overall mean ± SE		**15.75 ± 0.7**	**10.57 ± 0.8**	**8.74**	**0.001**
Sperm abnormalities of thawed semen					
Total abnormality (%) (Head, Mid piece, Tail)	Vasu	18.20 ± 0.7	12.00 ±0.5	11.87	0.001
	Ganesh	14.35 ± 0.8	11.00 ±0.7	4.70	0.01
	Pratap	20.83 ± 0.9	16.50 ±0.9	9.98	0.001
	Paras	17.47 ± 0.6	13.20 ± 0.8	4.96	0.01
	Sawan	14.67 ± 0.4	10.70 ± 0.5	5.55	0.001
	Baldev	18.67 ±0.4	15.10 ± 0.8	3.11	0.02
	Shiv	15.47 ± 0.5	14.60 ± 0.3	9.67	0.001
	Raj	20.88 ±0.9	15.70 ± 0.8	12.72	0.001
Overall mean ± SE		**17.56 ± 0.6**	**13.60 ± 0.6**	**5.99**	**0.001**