RESEARCH ARTICLE

Sexual Behavior and Semen Quality of Crossbred Bulls after Supplementation of Flaxseed and with Rubber Mat Floor

Samir Humeida Ali[1], Pawan Singh[2]*, Kotresh Prasad C[2], Mukesh Bhakat[2], Tushar Kumar Mohanty[1], Girish Panchabai[2], Ranjana Sinha[1]

Abstract

The experiment was conducted to study the effect of dietary supplementation of flaxseed and bedding material on sexual behavior, libido, and semen quality of crossbred bulls. For this, seventeen mature healthy breeding bulls were assigned to three groups as G1, G2, and G3 with 6, 5, 6 bulls, respectively. The bulls of G1 were fed as per standard diet (ICAR, 2013) and served as control. Bulls of G2 were fed as per standard diet G1 plus roasted flaxseed @ 1 kg/bull/day for 120 days, and bulls of G3 were provided rubber mat as bedding material and fed as per G2. The libido of the bulls was assessed by sexual behavior parameters and hormonal profile, and the seminal quality was examined. Significant ($P < 0.05$) changes were observed in sexual behavior of both the treatment groups as compared to control. Semen ejaculate volume, sperm concentration and total sperm output per ejaculate did not differ among the groups. Improved semen quality parameters*, viz.,* sperm motility, viability, morphology, and HOST reactivity were observed in the bulls supplemented with flaxseed and provided rubber bedding material. Testosterone, Luteinizing Hormone (LH), and estradiol hormones did not show any difference among the groups. It can be concluded that the dietary supplementation of roasted flaxseed alone and along with bedding material during winter season improved sexual behavior, libido, and the semen quality of crossbred bulls.

Keywords: Crossbred bulls, Roasted flaxseed, Rubber mat, Libido, Semen quality, Sexual behavior.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.3.14

Introduction

India is a leading milk producing country in the world. Artificial insemination using elite male germplasm is the major contributor in the growth of milk production. In India, Karan Fries cow is a cross of Holstein Friesian and Tharparkar developed at National Dairy Research Institute, Karnal. The production traits in animals are mostly influenced by genetics, but the reproduction traits are influenced by environment and plane of nutrition (Schillo *et al.,* 1992). The semen quality of bulls plays vital role in enhancing the conception rate and increasing the number of animals born in the herd (Tran *et al.,* 2016).

Nutritional and management intervention can play a key role to minimize the environmental influence. Flaxseed has an excellent source of alpha linolenic acid, energy and protein, essential amino acids, and minerals, which play vital role in synthesis of reproductive hormones (Petit *et al.,* 2004). Supplementation of n-3 PUFA improved semen quality in boar (Liu *et al.,* 2015), bull (Moallem *et al.,* 2015), buffalo bull (Tran *et al.,* 2016) and ram (Esmaeili *et al.,* 2012) by improving sperm fatty acid composition. Omega-3 fatty acids, especially docosahexaenoic acid (DHA), improve the sperm quality and protect the sperm against stress conditions by maintaining the membrane integrity and sperm viability (Gulliver *et al.,* 2012). Therefore, the present study was conducted with an objective to study the effect of flaxseed supplementation (Alpha-linolenic acid, the essential omega-3 fatty acid) and bedding materials on sexual behavior, libido, and semen quality of crossbred bulls during winter season.

[1]Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India

[2]Division of Livetsock Production Management, ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India

Corresponding Author: Pawan Singh, Division of Livetsock Production Management, ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India , e-mail: pawansinghdabas@gmail.com

How to cite this article: Ali, S.H., Singh, P., Prasad K.C., Bhakat, M., Mohanty, T.K., Panchabai, G., & Sinha, R. (2021). Sexual Behavior and Semen Quality of Crossbred Bulls after Supplementation of Flaxseed and with Rubber Mat Floor. Ind J Vet Sci and Biotech, 17(3): 65-68.

Source of support: Nil

Conflict of interest: None.

Submitted: 10/03/2021 **Accepted:** 23/06/2021 **Published:** 16/08/2021

Materials and Methods

The experiment was conducted on 17 mature healthy Karan Fries crossbred (Holstein Friesian × Tharparkar) breeding bulls under routine semen collection at Artificial Breeding Research Centre (ABRC), Karnal, Haryana, India for a period of 120 days during winter season from November 2016 to February 2017 after approval by IAEC. The average temperature humidity index (THI) observed in the experimental shed during study

period was 60.55°F ± 0.35, lowest being 55.23°F ± 0.24 during January and highest in November (68.52°F ± 0.63). The bulls were randomly divided into three treatment groups according to their age and body weight as group I (Control), group II (Flaxseed) and group III (Flaxseed + Bedding) with an average age of 53.50 ± 4.89, 46.17 ± 6.20 and 47.00 ± 5.18 months, respectively. These bulls were fed on standard (ICAR, 2013) diet to meet their nutrient requirements which was isocaloric and isonitrogenous in nature. The bulls of group I were kept as control without flaxseed supplementation, group II bulls were provided with roasted flaxseed @ 1 kg/bull/day and kept on concrete floor, while bulls under group III were supplemented with roasted flaxseed @ 1 kg/bull/day along with rubber mat as a floor bedding. Fresh water was provided *ad libitum* to the bulls throughout the experiment.

Semen collection was performed by experienced regular semen collector. Sexual behavior traits of each bull were recorded at the time of semen collection as per *Anzar et al.* (1993). Sexual behavior in terms of aggressiveness, active, dull and shyness, reaction time (seconds), dismounting time (seconds), total time taken (seconds), mating ability score, and sexual behavior score of every bull was recorded. Semen volume was recorded in graduated tubes and mass activity was assessed immediately after collection by gross swirl rating (GSR) of undiluted semen. Sperm morphology and viability were assessed by using eosin–nigrosin stain, and acrosome integrity by Giemsa stain. Hypo-osmotic swelling test was performed according to Correa and Zavos (1994).

Blood samples were collected fortnightly, and the plasma was separated and stored at -20°C for hormones analysis. Standardized procedures were followed for plasma testosterone (Sensitivity: 5.25 ng/L), LH (Sensitivity: 0.051 mIU/mL) and estradiol (Sensitivity: 2.53 pg/mL) hormones estimation (Bioassay Technology Laboratory, China).

The data obtained during experiment was tabulated and subjected to one way analysis of variance (Snedecor and Cochran, 1994) using the SPSS version 21.0® software. The means were tested for the significant difference by using Duncan's multiple range test.

Results and Discussion

Sexual Behavior of Bulls

Parameters related to sexual behavior of bulls are presented in Table 1. The results indicated significantly ($p < 0.05$) lower reaction time and total time taken to ejaculate in group III as compared to group I, however, the values in group II were statistically similar with other two groups. The significantly ($p < 0.05$) improved libido, mating ability and sexual behavior scores and reduced dismounting time was observed in group III bulls followed by group II and I. Similar observations

Table 1: Effect of flaxseed supplementation and bedding material on sexual behavior of Karan Fries bulls during winter season

Parameter	*Group I*	*Group II*	*Group III*
Reaction time (sec)	$37.11^{a} \pm 5.797$	$28.70^{ab} \pm 4.579$	$19.97^{b} \pm 2.409$
Libido score (%)	$66.11^{a} \pm 1.75$	$67.33^{a} \pm 1.85$	$71.67^{b} \pm 1.57$
Mating ability score (%)	$77.50^{a} \pm 1.885$	$80.33^{ab} \pm 2.061$	$88.61^{b} \pm 1.917$
Sexual behavior score (%)	$60.14^{a} \pm 9.523$	$68.89^{ab} \pm 8.55$	$71.94^{b} \pm 7.77$
Dismounting time (sec)	$5.11^{b} \pm 0.388$	$4.13^{ab} \pm 0.287$	$3.50^{a} \pm 0.286$
Total time taken to ejaculate (sec)	$94.06^{b} \pm 14.164$	$57.63^{ab} \pm 5.706$	$50.61^{a} \pm 4.336$

Means bearing different superscripts in a row differ significantly at level $p < 0.05$.

Table 2: Seminal attributes of fresh semen of crossbred bulls under different treatment groups during winter season

Parameters	*Group I*	*Group II*	*Group III*
Ejaculate volume (mL)	$4.81^{b} \pm 0.25$	$4.30^{a} \pm 0.23$	$5.03^{c} \pm 0.19$
Mass motility (0–5)	$2.17^{a} \pm 0.11$	$2.48^{b} \pm 0.06$	$2.70^{b} \pm 0.08$
Sperm concentration (10^{6}/mL)	780.72 ± 37.19^{A}	884.90 ± 33.98^{A}	1014.28 ± 29.52^{B}
Total sperm output	$3700.39^{a} \pm 242.52$	$3707.58^{a} \pm 239.78$	$5072.65^{b} \pm 230.66$
Motility (%)	$57.25^{a} \pm 1.70$	$63.60^{b} \pm 1.31$	$69.83^{c} \pm 1.07$
Live sperm (%)	$63.58^{a} \pm 0.75$	$68.76^{b} \pm 1.44$	$74.40^{c} \pm 1.08$
HOST (%)	$58.67^{a} \pm 2.112$	$68.66^{b} \pm 1.18$	$75.45^{c} \pm 0.96$
Acrosome integrity (%)	$76.08^{a} \pm 10.89$	$80.06^{b} \pm 7.19$	$83.93^{c} \pm 5.34$
Head abnormality (%)	$4.37^{b} \pm 0.52$	$3.14^{a,b} \pm 0.44$	$2.27^{a} \pm 0.20$
Tail abnormality (%)	$3.70^{b} \pm 0.23$	$3.06^{a} \pm 0.29$	$3.15^{a} \pm 0.29$
Midpiece abnormality (%)	$5.08^{b} \pm 0.34$	$4.58^{b} \pm 0.32$	$3.90^{a} \pm 0.27$
Total abnormal sperm (%)	$13.17^{b} \pm 0.72$	$10.64^{a} \pm 0.651$	$9.22^{a} \pm 0.57$

Means bearing different superscripts in a row differ significantly at level $p < 0.05$) (small) and at $p < 0.01$ (capital).

Table 3: Plasma testosterone, luteinizing hormone and estradiol hormone levels of bulls during winter season

Parameter	*Group I*	*Group II*	*Group III*
Testosterone (ng/mL)	3.72 ± 0.42	4.12 ± 0.31	3.92 ± 0.26
Luteinizing hormone (IU/mL)	8.62 ± 0.43	9.21 ± 0.21	8.84 ± 0.38
Estradiol hormone (pg/mL)	129.33 ± 29.38	161.96 ± 26.64	151.08 ± 32.18

were reported by Pal *et al*. (2004). We observed a positive trend of better libido score in crossbred bulls with flaxseed supplementation, whilst observations reported by Pal and Chakravarty (2004) were lower than present ones. It might be due to feeding and management.

Seminal Attributes of Fresh Neat Semen

The mean values of ejaculate volume and mass motility observed were significantly ($p < 0.05$) higher in bulls of group III than in group II and I (Table 2). Similarly, the sperm concentration per mL, total sperm output and sperm motility were found significantly ($p < 0.05$) higher in group III as compared to group I and/or II. Present findings for sperm concentration per mL were comparatively lower than mean values reported by Soren *et al.* (2016) and Kumar *et al.* (2016), whereas Mallick *et al.* (2016) found lower sperm concentration/mL in HF crosses (KF) than values observed in the present study. Khan *et al.* (2015) found higher sperm concentration in flaxseed supplemented HF bulls' group (1037.40 ± 80.9.09 million) as compared to Jersey and Friesian-Sahiwal bulls.

The percentages of live sperm, HOST reacted sperm count and acrosomal integrity differed significantly ($p < 0.05$) among three treatment groups, being highest in group III followed by group II and group I. The live sperm per cent obtained in group III bulls agreed with the earlier findings of Mallick *et al.* (2016). However, slightly higher mean values were reported by Khan *et al.* (2015) and Moallem *et al.* (2015). The incorporation of PUFA in the diet can cause alteration of fatty acid profile of sperm plasma membrane and results in improved sperm quality (Moallem *et al.,* 2015). The head, tail and mid-piece abnormalities were found to be significantly ($p < 0.05$) lower in group III as compared to group I and II.

The present findings of acrosome integrity are similar with previous reports of Soren *et al.* (2016). However, Kumar *et al*. (2016) reported high acrosome integrity in fresh semen sample of Sahiwal bulls. Significantly higher acrosome integrity was observed in group III followed by Group II and Group I. The variations in acrosome integrity were observed in the present investigation may be due to different treatment groups and better improvement was found in group III. The percentage of HOST reactive sperm obtained in bulls of flaxseed supplementation and bedding group corroborated with the findings of Mehmood (2009). Positive effect of flaxseed supplemented diet was obtained by reducing percent total sperm abnormality in semen samples. The higher percentage of total sperm abnormality obtained in control group agreed with the findings of Tran *et al*. (2016), who fed prilled fat for control buffalo bulls and protected flaxseed oil to the treatment buffalo bulls.

Hormonal Profile

The level of testosterone, estradiol, and luteinizing hormone (LH) did not vary among the groups during the experimental period (Table 3). The results did not show any significant effect of flaxseed supplementation with or without rubber mat flooring in crossbred bulls. However, the estradiol concentration was higher in flaxseed supplemented group compared to control group I. The results agree with Li *et al.* (2017), who reported higher estradiol concentration (19.50 ± 1.27 pg/mL vs. 14.88 ± 0.67 pg/mL) in rams supplemented with dietary linseed oil during peri-pubertal period as it stimulates steroidogenesis and testis development in rams. This might be due to breed and growing age as compared to mature bulls. Unsaturated fatty acids have been reported to improve steroidogenesis *in vivo* through steroidogenic acute regulatory (StAR) protein expression (Hughes *et al.,* 2011) and altering the function of transcription factors (Wathes *et al.,* 2007). However, no such effect of flaxseed supplement was noted in present study on mature crossbred bulls.

Conclusion

The dietary supplementation of roasted flaxseed @ 1 kg / bull/day and rubber mat floor as bedding material during winter season significantly improved sexual behavior and semen quality parameters of crossbred bulls. Therefore, it can be recommended that crossbred breeding bulls may be provided roasted flaxseed in their diets along with comfortable flooring during winter to get better libido and harvest quality semen throughout the season.

Acknowledgments

The authors are grateful to Director, ICAR-National Dairy Research Institute for funding the project and Artificial Breeding Research Centre of ICAR - NDRI for providing the facilities for conducting this study.

References

Adwani, R. (1992). Effect of certain management practices on temperament and libido of young bulls. *M S. Thesis.* National Dairy Research Institute (Deemed University), Karnal, India.

Anzar, M., Ahmad, M., Nazir, M., Ahmad, N., & Shah, I.H. (1993). Selection of buffalo bulls: Sexual behavior and its relationship

to semen production and fertility. *Theriogenology, 40*, 1187-1198.

Correa, J.R., & Zavos, P.M. (1994). The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology, 42*(2), 351-360.

Esmaeili, V., Shahverdi, A.H., Alizadeh, A.R., Alipour, H., & Chehrazi, M. (2012). Saturated, omega-6 and omega-3 dietary fatty acid effects on the characteristics of fresh, frozen- thawed semen and blood parameters in rams. *Andrologia, 46,* 42-49.

Gulliver, C.E., Friend, M.A., King, B.J., & Clayton, E.H. (2012). The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. *Animal Reproduction Science, 131*(1), 9-22.

Hughes, J., Kwong, W.Y., Li, D., Salter, A.M., Lea, R.G. and Sinclair, K.D. (2011). Effects of omega-3 and -6 polyunsaturated fatty acids on ovine follicular cell steroidogenesis, embryo development and molecular markers of fatty acid metabolism. *Reproduction, 141*, 105-118.

ICAR (2013). Nutrients requirements of cattle and buffalo. Indian Council of Agricultural Research, New Delhi (India).

Khan, H., Qureshi, S.M., Khan, I., Rehman, S.U., Mehsud, T., Ullah, I., Afrab, M., & Rehman, F.U. (2015). Dietary flaxseed supplementation effect on bovine semen quality parameters. *Veterinaria, 3*, 9-13.

Kumar, D., Kumar, P., Singh, P., Yadav, S.P., & Yadav, P.S. (2016). Assessment of sperm damages during different stages of cryopreservation in water buffalo by fluorescent probes. *Cytotechnology, 68,* 451-458.

Li, W., Tang, D., Li, F., Tian, H., Yue, X., Li, F., Weng, X., Sun, W., Wang, W., & Mo, F. (2017). Supplementation with dietary linseed oil during peri-puberty stimulates steroidogenesis and testis development in rams. *Theriogenology, 102,* 10-15.

Liu, Q., Zhou, Y.F., Duan, R.J., Wei, H.K., Jiang, S.W., & Peng, J. (2015). Effects of dietary n-6:n-3 fatty acid ratio and vitamin E on semen quality, fatty acid composition and antioxidant status in boars. *Animal Reproduction Science, 162*, 11-19.

Mallick, S., Agarwal, A., & Prakash, B.S. (2016). Seasonal changes in semen quality and correlation with plasma hormone profiles in Karan Fries bulls. *Biological Rhythm Research, 47*(6), 967-974.

Mehmood, A., Anwar, M., & Saqlan, S.M. (2009). Motility, acrosome integrity, membrane integrity and oocyte cleavage rate of sperm separated by swim-up or Percoll gradient method from frozen–thawed buffalo semen. *Animal Reproduction Science, 111*, 141-148.

Moallem, U., Neta, N., Zeron, Y., Zachut, M., & Roth, Z. (2015). Dietary α-linolenic acid from flaxseed oil or eicosapentaenoic and docosahexaenoic acids from fish oil differentially alter fatty acid composition and characteristics of fresh and frozen-thawed bull semen. *Theriogenology, 83*, 1110-1120.

Pal, A., Chakravarty, A.K., & Bhattacharya, T.K. (2004). Association of genetic polymorphism of growth hormone gene with some sexual behavioral characteristics in dairy animals. *In Proceedings of 4th Indian Veterinary Congress,* IVRI, Izatnagar, India. pp. 3-5.

Pal, A., & Chakravarty, A.K. (2004). Marker genotype of growth hormone gene for assessing the libido of crossbred dairy bulls. *National Symposium on Conservation and Propagation of Indigenous Breeds of Cattle and Buffaloes*. February 26-28, 2004, Pantnagar, Uttaranchal, India.

Petit, H.V., Germiquet, C., & Lebel, D. (2004). Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. *Journal of Dairy Science, 87*(11), 3889-3898.

Schillo, K.K., Hall, J.B., & Hileman, S.M. (1992). Effects of nutrition and season on the onset of puberty in the beef heifer. *Journal of Animal Science, 70***,** 3994-4005.

Snedecor, G.W., & Cochran, W, G. (1994). *Statistical Methods*. 8th Ed., Iowa State University Press. Ames, Iowa, USA, p.314.

Soren, S., Singh, S.V., & Kumar, A. (2016). Influence of season on semen quality in Karan Fries (Tharparkar × Holstein Friesian) bulls. *Journal of Animal Research, 6*(2), 121-125.

Tran, L.V., Malla, B.A., Sharma, A.N., Sachin, K., Tyagi, N., & Tyagi, A.K. (2016). Effect of omega-3 and omega-6 polyunsaturated fatty acid enriched diet on plasma IGF-1 and testosterone concentration, puberty and semen quality in male buffalo. *Animal Reproduction Science, 173*, 63-72.

Wathes, D.C., Abayasekara, D.R., & Aitken, R.J. (2007). Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction, 77,* 190-201.