

Dietary Flaxseed supplementation effect on bovine semen quality parameters

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Abstract

The objective of present was to determine the effect of dietary Flaxseed supplementation (rich source of Omega 3 fatty acid) on the bull sperm quality. Three different cattle breeds (i.e. Holstein Friesian, Jersey, Friesian-Sahiwal crossbred) were used and Flaxseed was given in ration for nine weeks. The fresh semen quality parameters viz seminal volume, sperm concentration, mass motility and percent motility were observed every week. The results showed that the sperm count was significantly ($p < 0.05$) affected by the Flaxseed supplementation and observed higher in Holstein Friesian followed by Jersey breed. Similarly, the mass motility was also found highest in Holstein Friesian followed by Jersey. However, the seminal volume remained non-significant ($p > 0.05$) among various treated groups. Based upon the results it can be concluded that linseed supplementation might improves semen quality in bovine bulls.

Keywords: Artificial insemination, bull fertility, breeds, Flaxseed supplementation

Received: 07 May, 2015

Revised: 9 September 2015

Accepted: 03 October, 2015

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To Cite This Manuscript: Khan H, Qureshi SM, Khan I Rehman SU, Mehsud T, Ullah I, Afrab M, Rehman FU. Dietary flaxseed supplementation effect on bovine semen quality parameters. Veterinaria 2015; 3: 9-13.

Introduction

Economically bull fertility plays a key role in cattle artificial insemination (AI) industry. The bull fertility can be evaluated by the semen quality parameters. There are many factors that affect the semen quality i.e. feeding, management practices, climatic factors and genetic makeup [1, 2]. In males, the semen quality is affected by environmental temperature which alter the spermatogenesis and hence influences the reproduction process [3]. Nutrition also plays vital role in regulating the reproductive events. It directly influences the process of oocyte and spermatozoa maturation, ovulation and fertilization, and/or decreases the metabolite and hormones level necessary for reproduction that indirectly alters the said reproductive process [4].

Flaxseed oil contains 58 % linolenic acid which is excellent source of antioxidant and improve animals health [5]. The botanical name of flax is *Linum usitatissimum* from the family Linaceae.

Flaxseed also known as *alsi* or linseed is an excellent source of alpha linolenic acid and has been shown to increase tissue concentrations of both alpha linolenic acid and eicosapentaenoic acid, which are involved in synthesis of important reproductive hormones [6]. 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 [7].

Flax is rich in fat, protein and dietary fiber. An analysis of brown Canadian flax averaged 41% fat, 20% protein, 28% total dietary fibre, 7.7% moisture and 3.4% ash that is the mineral-rich residue left after samples are burned [8]. The vertebrates cannot synthesized omega-3 and omega-6 series of polyunsaturated fatty acids (PUFAs) and hence must be provided by the diet, either in the form of short chain 18 carbon derivatives found in plants or in the form of long chain 20-22 carbon containing 2-6 double bonds derivatives found in animal tissues [9]. Therefore the omega-3 rich food must be supplemented into their feed to fulfil the animal requirement. The Flaxseed has been extensively investigated for the fertility enhancement [5, 10, 11]. In this context, the current study was designed to investigate the effect dietary Flaxseed supplementation on sperm quality parameters in different dairy cattle bull breeds.

Materials and Methods

Selection of animals and supplementation

A total of twelve cattle bulls (age of 3-5 years, regular sperm donors) Holstein Friesian (HF; n=04), Jersey (JR; n=04) and Friesian-Sahiwal crossbred (FS; n=04) dairy breeds were selected. Two animals from each breed were kept in

treatment (FL-L) and two in control group (FL-0). Water was provided *ad-labium* and FL-L group each animal was fed 6 kg concentrate, 6 kg wheat straw and 40 kg green fodder supplemented with 50 gm Flaxseed (purchased from local market), whereas FL-0 was kept as control without supplementation. Flaxseed were mixed in concentrate and then feed to FL-L treatment group. The initial two week time period was considered as adaptation period, and at week three sampling was started till the end of week eleven.

Table 1: Ingredients of semen extender used for semen dilution

Ingredients	Quantity used
Tris(hydroxymethyl) aminomethane	0.73 mM
Citric acid	0.25 mM
Fructose	0.20 mM
Streptomycin	6.4 mM
Benzyl penicillin	50,000 IU
Egg yolk	200 ml
Glycerol	154 mM
Total	270 ml

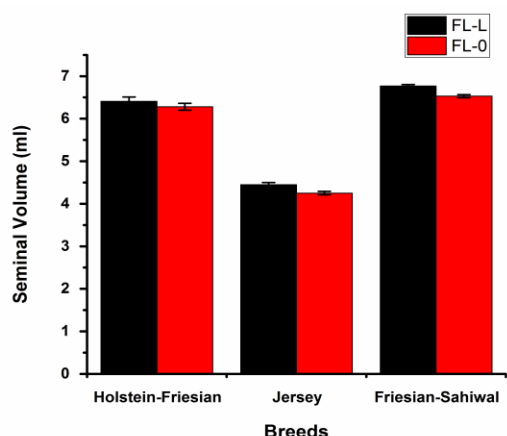


Fig. 1: Effect of Flaxseed supplementation on the seminal volume in various cattle breeds as semen quality parameter

Here in this figure, FL-L stands for Flaxseed supplemented cattle breed whereas FL-0 represent control group without supplementation of Flaxseed

Extender Preparation

The semen extender was prepared as according to the procedure mentioned earlier [12], briefly the composition of semen extender is showed in table 1. All chemicals were purchased from Sigma-Aldrich Co. LLC. St Louis MO 63178 USA, except Glycerol that was obtained from Merck Millipore Co. Darmstadt Germany.

Semen Collection

For semen collection the procedure was followed reported earlier by Rehman and Qureshi [13]. Briefly, the semen was collected twice a week from twelve bulls by artificial vagina having 42 °C temperature and 30 cm size. Semen was collected early in the morning before the dawn. Teaser bull was used for semen collection. The collecting tubes were immediately transferred to water bath at of 35 °C. The collected semen samples were further examined for the following parameters.

Volume (%)

Seminal volume was measured through graduated test tube used for the semen collection.

Mass Motility

Mass motility means the overall motility of the spermatozoa. It was determined through light microscope [14]. A drop of fresh collected semen was placed on pre warmed slide without cover slip. The swirls/waves of the spermatozoa were recorded and graded as very good, good, fair and poor depending upon the darkness and speed of the swirls and eddies. Mass motility of the spermatozoa is however affected by factors like concentration of the sperm cells, percentage of the motile spermatozoa and speed of the sperm cells. The waves/swirling of mass motion were suppressed if any one of the above mentioned factor(s) were present. The evaluation scale designed was as follows; Very good (++++ = when dark, rapid waves/ swirls and eddies were present; Good (+++) = when dark slower swirls and eddies were present; Fair (++) = when prominent individual sperm cell motion were present but no swirls/waves; Poor (+) = when individual cell motion was less or absent.

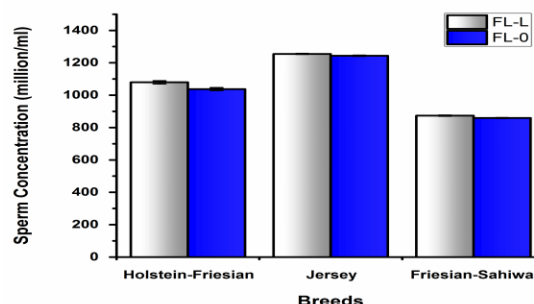


Fig. 2: Effect of Flaxseed supplementation on sperm concentration in various cattle breeds

Here in this figure, FL-L stands for Flaxseed supplemented cattle breed whereas FL-0 represent control group without supplementation of Flaxseed

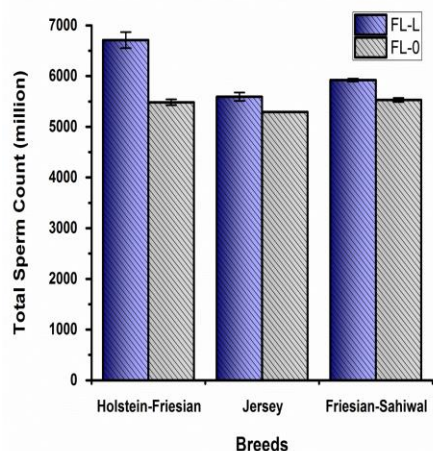


Fig. 3: Effect of Flaxseed supplementation on total sperm count in various cattle breeds

Here in this figure, FL-L stands for Flaxseed supplemented cattle breed whereas FL-0 represent control group without supplementation of Flaxseed.

Sperm Concentration

Concentration of the spermatozoa was calculated through haemocytometer [14]. Semen was diluted 200 times with 3% sodium chloride solution. Three g NaCl was mixed with 100 ml distal water. Then 25 micro liters (μ l) of this solution was removed and 25 μ l fresh semen was added to achieve 200 times dilution. Through micropipette one drop of this solution was placed at the edge of haemocytometer. Calculation was made in 5 large chambers from A to E which were examined under simple microscope at 40 X. Chamber E was the central and A to D were taken as marginal chambers. The average of the number of sperm cells was calculated by counting both side chambers of the haemocytometer. The depth of the groove was 0.1 mm and the counted spermatozoa were placed in the following formula:

$$\text{Number of sperm cells/ml} = \frac{\text{Number of cells counted} \times \text{Dilution} \times 1000}{0.004 \times \text{Number of chambers counted}}$$

0.004* x Number of chambers counted

*0.004 is the volume in one large chamber

Statistical analysis

The collected data was arranged in excel program and statistically analyzed through SPSS version.18 for t-test.

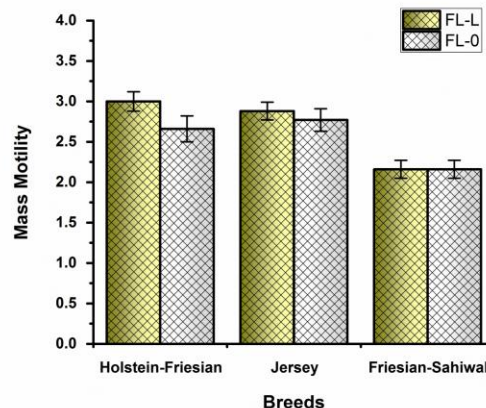


Fig. 4: Effect of Flaxseed supplementation on sperm mass motility in various cattle breeds

Here in this figure, FL-L stands for Flaxseed supplemented cattle breed whereas FL-0 represent control group without supplementation of Flaxseed.

Results

The present study was designed with the aim to determine the effects of dietary supplementation of flaxseed on fresh quality and production in different dairy cattle breeds.

The effect of Flaxseed on semen quality was found non-significant among all groups. (Fig 1) However the sperm concentration mean was found relatively higher in Holstein Friesian Flaxseed treated group (i.e. 1079.40 ± 10.01 (SE)) as compared to control group (1037.80 ± 9.09 (SE)) whereas other breeds remained non affected. (Fig 2)

The total sperm count was found significantly higher ($p < 0.05$) in all breeds of IL-L group i.e. HF, Jersey and FS 6710.2 ± 159.75 (SE), 5592.8 ± 83.940 (SE), 5922.7 ± 28.259 (SE) respectively, as compared to control group (5484.4 ± 58.265 (SE), 5294.2 ± 62.084 (SE), 5529.2 ± 38.798 (SE), respectively. (Fig 3)

Similarly, the mass motility also significantly ($p < 0.05$) affected by Flaxseed supplementation in HF (3.00 ± 0.12 (SE)) followed by Jersey (2.88 ± 0.11 (SE)) as compare to control 2.66 ± 0.16 (SE), 2.77 ± 0.14 (SE). Whereas non-significant effect of Flaxseed supplementation was found in FS breed. (Fig 4)

Discussion

In the present study dietary supplementation of flaxseed as n-3 source did not improve the seminal volume which are in agreement with earlier reports in buffaloes[15], in bulls[11], in chicken[16], and

in turkey [17]. However, the Flaxseed (FL-L) feeding as a source of n-3 fatty acids produced higher semen concentration as compared to control group (FL-0). The same results were discussed by Fair *et al*, who supplementation of fish oil to rams and resulted in higher semen concentration [18]. Our results are in contrast with Adeel *et al.*, 2009 in buffaloes and Hamid *et al.*, 2010 in bull who studied that feeding omeg-3 fatty acids had no effects on total sperm output. This difference may be attributed to various source of omega-3 fatty acids as Hamid., *et al* 2010 used rumen protected neutraceuticals as source of omega-3 while Adeel *et al.*, 2009 used sunflowers as source of omega-3 fatty acids in buffalo bulls. Nevertheless, in current study we used commercially available Flaxseed.

Similarly, feeding a source of omega-3 fatty acids leads to a higher sperm concentration and total production in boar [19] and stallion[10, 20]. Various studies also reported the effect of DHA on sperm concentration and total production that may be related to the proportion of DHA and Docosapentaenoic acid C22:5n-6 (DPA) in semen of boar and stallion. Our results demonstrated that feeding of flaxseed (FL-L) as source of n-3 fatty acids had good positive effect on the bovine bull semen quality parameters, which is in corroboration with already reported various findings in cattle bull [11], man [21], boar [22] and buck [23, 24]. These studies also reported a significant correlation between dietary supplementation of n-3 and number of motile spermatozoa. Omega-3 supplementation increased phospholipids into spermatozoa and modify the fatty acids profile of membrane.

All the above findings are vouching the effect of Flaxseed as dietary source of omga-3 that affect semen quality in various species and are in agreement with our findings for bovine various breeds semen quality enhancement.

Conclusion

In summary, effect of Flaxseed supplementation on the various cattle breeds fresh semen quality by increasing the sperm cell concentration, sperm count, and mass motility. Among various breed bulls the most improved semen quality parameters post Flaxseed feeding were observed in Holstein Friesian bulls followed by Jersey.

References

- [1] Qureshi M. A review of Artificial Insemination status in NWFP. Proc National Seminar on Livestock Breed Improvement, present status and its future 2004.
- [2] Rehman F, Qureshi M, Khan R. Effect of soybean based extenders on sperm parameters of holstein-friesian bull during liquid storage at 4°C. Pakistan J Zool 2014; 46:185-9.
- [3] Casu S, Cappai P, Naitana S. Effects of high temperatures on reproduction in small ruminants. EAAP Publication (Netherlands). 1991.
- [4] Wathes DC, Abayasekara DRE, Aitken RJ. Polyunsaturated fatty acids in male and female reproduction. Biol Reprod 2007; 77: 190-201.
- [5] Mourvaki E, Cardinali R, Dal Bosco A, Corazzi L, Castellini C. Effects of flaxseed dietary supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. Theriogenology 2010; 73: 629-37.
- [6] Petit H, Germiquet C, Lebel D. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J Dairy Sci 2004; 87: 3889-98.
- [7] Gulliver C, Friend M, King B, Clayton E. The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim Reprod Sci 2012; 131: 9-22.
- [8] Dean JR. 15 Current market trends and economic importance of oilseed flax. Flax: the genus Linum. 2003: 275.
- [9] Cook HW. Fatty acid desaturation and chain elongation in eucaryotes. Biochemistry of lipids, lipoproteins and membranes. 1991: 141-69.
- [10] Brinsko SP, Varner DD, Love CC, Blanchard TL, Day BC, Wilson ME. Effect of feeding a DHA-enriched nutraceutical on the quality of fresh, cooled and frozen stallion semen. Theriogenology. 2005; 63: 1519-27.
- [11] Gholami H, Chamani M, Towhidi A, Fazeli MH. Improvement of semen quality in holstein bulls during heat stress by dietary supplementation of omega-3 fatty acids. Int J Fertil Steril. 2011; 4: 160.
- [12] Rehman FU, Zhao C, Shah MA, Qureshi MS, Wang X. Semen Extenders and Artificial Insemination in Ruminants. Veterinaria. 2013; 1: 1-8.
- [13] Rehman Fu. Substitution of animal protein source with plant protein in semen extenders of various cattle breeds: Khyber Pakhtunkhwa Agricultural University; 2012.
- [14] Fawad Ur Rehman MSQ, Sajid Ur Rehman, Ikramullah Khan, Hafiz Abdul Majid, Muhammad Aftab. Conventional semen processing technique and breeding soundness of various cattle breed bulls used in sub-tropical conditions. Sci Lett. 2013; 1: 9-13.
- [15] Adeel M, Ijaz A, Aleem M, Rehman H, Yousaf M, Jabbar M. Improvement of liquid and frozen-thawed semen quality of Nili-Ravi buffalo bulls (Bubalus bubalis) through supplementation of fat. Theriogenology 2009; 71: 1220-5.
- [16] Cerolini S, Zaniboni L, Maldjian A, Gliozzi T. Effect of docosahexaenoic acid and α -tocopherol enrichment in chicken sperm on semen quality, sperm lipid

- composition and susceptibility to peroxidation. *Theriogenology* 2006; 66: 877-86.
- [17] Zaniboni L, Rizzi R, Cerolini S. Combined effect of DHA and α -tocopherol enrichment on sperm quality and fertility in the turkey. *Theriogenology* 2006; 65: 1813-27.
- [18] Fair S, Doyle D, Diskin M, Hennessy A, Kenny D. The effect of dietary n-3 polyunsaturated fatty acids supplementation of rams on semen quality and subsequent quality of liquid stored semen. *Theriogenology* 2014; 81: 210-9.
- [19] Estienne M, Harper A, Crawford R. Dietary supplementation with a source of omega-3 fatty acids increases sperm number and the duration of ejaculation in boars. *Theriogenology* 2008; 70: 70-6.
- [20] Castellano C-A, Audet I, Bailey J, Chouinard P, Laforest J-P, Matte J. Effect of dietary n-3 fatty acids (fish oils) on boar reproduction and semen quality. *J Anim Sci* 2010; 88: 2346-55.
- [21] Conquer JA, Martin JB, Tummon I, Watson L, Tekpetey F. Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. *Lipids* 2000; 35: 149-54.
- [22] Rooke JA, Shao CC, Speake BK. Effects of feeding tuna oil on the lipid composition of pig spermatozoa and in vitro characteristics of semen. *Reproduction*. 2001; 121: 315-22.
- [23] Dolatpanah M, Towhidi A, Farshad A, Rashidi A, Rezayazdi A. Effects of dietary fish oil on semen quality of goats. *Asian Aust J Animal Sci*. 2008; 21:29.
- [24] Samadian F, Towhidi A, Rezayazdi K, Bahreini M. Effects of dietary n-3 fatty acids on characteristics and lipid composition of ovine sperm. *Animal* 2010; 4: 2017-22.