Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.053

RESEARCH ARTICLE

# Effect of Dietary Vitamin E and Selenium Supplementation on Physiological Responses and Reproductive Performance in Holstein Friesian Bulls during Humid Hot Summer

Mahboob Ahmed Butt[1], Muhammad Qamer Shahid*[1], Jalees Ahmad Bhatti[2] and Anjum Khalique[3]

[1]Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore, Ravi Campus
[2]Department of Animal Sciences, College of Veterinary and Animal Sciences, Jhang
[3]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Ravi Campus
*Corresponding author: qamar.shahid@uvas.edu.pk

ARTICLE HISTORY (19-053)

Received: February 19, 2019
Revised: March 15, 2019
Accepted: March 20, 2019
Published online: April 06, 2019

**Key words:**
Holstein Friesian bulls
Oxidative heat stress
Selenium
Semen traits
Vitamin E

**ABSTRACT**

The objective of this study was to assess the effect of dietary vitamin E (Vit. E) and Selenium (Se) supplementation on physiological and reproductive performance of bulls during humid hot summer in Pakistan. Eighteen Holstein Friesian bulls balanced by age and weight were divided into three treatment groups: 1) CTL, basal diet without Se and Vit. E supplementation; 2) SeS, basal diet supplemented with 3 g Selemax® (Biorigin, USA); 3) ESeS, basal diet with 3 g Selemax® and Vit. E @ 6000 IU/day). Basal diet consisted of 50% green fodder, 25% wheat straw and 25% concentrate on dry matter basis. Diets were offered for 13 weeks from July 2016 to October 2016 having temperature-humidity index ranging from 83 to 87. Data obtained were subjected to repeated measures ANOVA. Supplementation of Vit. E and Se did not affect dry matter intake, water intake, respiration rate, rectal temperature, and pulse rate. The ESeS treatment had higher sperm motility% compared to CTL and SeS ($P<0.05$). The amplitude of lateral head displacement (ALH) was higher in SeS and ESeS compared to CTL ($P<0.05$). Straightness was higher in SeS group compared to CTL and ESeS. However, Vit. E and Se had no effect on other semen traits including semen volume, concentration, progressive motility, DNA integrity, plasma membrane integrity, and live to dead ratio. Blood testosterone concentration was significantly higher in SeS and ESeS groups compared to CTL. Blood glucose and blood urea nitrogen were similar among the treatments. Current results indicated that supplementation of Vit. E and Se improved only sperm motility, ALH, straightness and testosterone level during humid hot summer in Holstein Friesian bulls.



**To Cite This Article:** Butt MA, Shahid MQ, Bhatti JA and Khalique A, 2019. Effect of dietary vitamin E and selenium supplementation on physiological responses and reproductive performance in Holstein Friesian bulls during humid hot summer. Pak Vet J, 39(4): 593-597. http://dx.doi.org/10.29261/pakvetj/2019.053

## INTRODUCTION

Pakistan has admirable breeds of cattle with a sizeable number of non-descriptive cows (Khan *et al.*, 2008). Cross-breeding of non-descript cows with exotic dairy breeds especially Holstein Friesian, lead the establishment of semen production units in the country. Geographically, Pakistan is located in the subtropical region of the world. The longer summer season with high ambient temperature and relative humidity are major factors affecting livestock performance in the area. Heat stress during summer negatively affects reproductive performance of breeding bulls.

Also, heat stress induces oxidative stress in animals (Mirzad *et al.*, 2018). During oxidative stress, concentration of certain free radicals like "reactive oxygen/nitrogen/chlorine species" increases and causes cellular damage (Halliwell and Whiteman, 2004). The presence of reactive oxygen species was associated with poor sperm concentration, motility, morphology (Agarwal *et al.*, 1994), altered spermatogenesis, acrosome reaction and sperm-oocyte fusion (Cocuzza *et al.*, 2007), thereby decreasing fertilization rates (Lewis and Aitken, 2005).

Vitamin E (Vit. E) and Selenium (Se) are documented antioxidants (Domosławska *et al.*, 2018). Vit. E has also been reported to reduced testicular oxidative stress (Tvrdá *et al.*, 2016) and improve sperm quality in bulls under testicular heat stress (Losano *et al.*, 2018). Vit. E and Se have conjoint effect on many biological processes including reproduction (Koyuncu and Yerlikaya, 2007; Zubair *et al*., 2018), spermatogenesis and semen quality (Yousef *et al*., 2003). Vit. E and Se also improved physiological measures in sheep during heat stress (QureShi *et al*., 2017). There is a need to further explore the use of Vit. E and Se in exotic bulls reared in subtropical environment.

The objective of current study was to investigate the effect of Vit. E and Se on physiological and reproductive performance of Holstein Friesian bulls during humid hot summer.

## MATERIALS AND METHODS

**Study animals and treatment groups:** The present study was conducted at government semen production unit, Renala Khurd, Distt. Okara, Punjab, Pakistan (30.8874377, 73.5549005). Eighteen mature Holstein Friesian bulls balanced by body weight (898.8±137.3 kg; Mean ± SD) and age (4 to 6 years) were divided into three treatment groups;1) CTL, basal diet without mineral and vitamin supplementation; 2) SeS, basal diet supplemented with 3 g Selemax® (Biorigin, USA); and 3) ESeS, basal diet with 3 g Selemax® and vitamin E @ 6000 IU/day). The supplementation of Se and Vit. E was based on NRC (2001) and Velasquez-Pereira *et al*. (1998), respectively. The supplementation was top dressed on concentrate feed. The trial lasted for 13 weeks starting from July 11, 2016 till October 09, 2016.

**Animal housing and management:** The bulls were kept in individual pens of 11 ft wide and 63 ft long with 23 ft long covered area. Each pen had separate water trough and feeding area. Animals were fed at the rate of 2% bodyweight on dry matter (DM) basis (NASEM, 2016), where 1% DM was provided through green fodder/silage, 0.5% through wheat straw, and 0.5% through concentrate feeding. The concentrate composition is presented in Table 1. All the animals were given 7 days of acclimatization period before the start of data collection. Deworming and vaccination were done according to standard procedure of semen production unit.

**Meteorological measures:** Ambient temperature (T, ºC) and relative humidity (RH, %) were recorded at 1400 h daily using hygrometer. The recording time was selected because around that time the ambient temperature was highest. Temperature-humidity index (THI) was calculated according to the following equation (Kelly and Bond, 1971):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

**Feed and water intake:** Feed was weighed and offered daily at 1000 h and orts were collected the next morning to measure daily intake. Each water trough was filled daily with measured quantity and intake was recorded.

**Physiological measures:** Physiological measures were recorded on daily basis at 1230 to 1430 h. Rectal temperature (RT) was recorded with mercury thermometer, respiration rate (RR) was recorded by counting the movement of flank region, and pulse rate (PR) was recorded from the coccygeal artery.

**Table 1:** Ingredients and nutrient composition of concentrate diet

| Ingredients | Inclusion level (%) |
|---|---|
| Wheat bran | 40 |
| Maize oil cake | 5 |
| Maize grain | 20 |
| Wheat grain | 10 |
| Rice paddy | 8 |
| Corn gluten 30% | 5 |
| Canola meal | 5 |
| Soybean meal | 5 |
| Molasses | 1 |
| Premix | 1 |
| Nutrient composition | Percentage (%) |
| Dry matter | 93.24 |
| Crude protein | 14.96 |
| Ether extract | 4.25 |
| Ash | 3.87 |
| Crude fiber | 3.05 |

**Semen collection and semen traits:** Semen was collected twice a week between 0415 to 0530 h from each bull and evaluated on fortnightly basis. Semen volumes for each ejaculate from individual bull along with concentration (by using photometer) were recorded and processed using extender Triladyl® (Minitube, Germany). Thawed semen was evaluated for the percent motility, progressive motility, lateral head displacement and straightness by using Computerized External Real Imaging Optic System (Himilton Thorne Biosciences, USA). Live and dead count was determined by using eosin and nigrosin stain (Mortimer *et al*., 2015). Plasma membrane integrity was determined by following the method adopted by Tartaglionea and Ritta (2004). Formal citrate solution (1%) was used to assess the normal apical ridge percentage through phase contrast microscope as described by Khan and Ijaz (2007), and percent DNA integrity was calculated by following the method adopted by Tejada *et al*. (1984).

**Blood biochemistry:** Blood samples of about 5 ml were taken fort-nightly from jugular vein of each experimental bull in gel vacutainer and the separated serum was stored at 4ºC for further analysis. The analysis was done for glucose by using kit (BioMed Diagnostics, Hannover, Germany), blood urea nitrogen (Urea/BUN by linear chemicals, Barcelona, Spain), and testosterone by enzyme immune assay test kit (BioCheck, Inc. Foster City, CA) at biochemistry lab University of Veterinary and Animal Sciences (UVAS), Ravi Campus.

**Statistical analysis:** The recorded data were subjected to statistical analysis through analysis of variance (ANOVA) using Mixed Procedure of SAS (SAS University Edition; SAS Institute Inc., Cary, NC). Treatments were considered as fixed effect and base line variables of semen quality were used as covariate. The dependent variables recorded multiple times on same animal were subjected to repeated measures ANOVA. Dunnett's test was applied to compare the treatment means with CTL. Statistical significance was set at $P<0.05$ and a tendency at P between 0.05 and 0.1.

## RESULTS

**Meteorological measures:** The THI values for the study period of 13 weeks are presented in Fig. 1. The THI was in the rage of 87-85 in first 6 weeks of study and then slightly reduced to around 83 in next 7 weeks (Fig. 1).

**Table 2:** Effect of Vitamin E and Selenium supplementation on feed intake, water intake and physiological measures of Holstein Frisian bulls during summer

| | Treatment[1] | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|
| Variables | CTL | SeS | ESeS | SEM[3] | Treat | *CTL vs SeS | *CTL vs ESeS |
| Feed intake (kg) | 16.56 | 17.44 | 17.34 | 0.74 | 0.6630 | 0.4148 | 0.4677 |
| Water intake (L) | 35.08 | 33.27 | 34.40 | 2.73 | 0.9694 | 0.8621 | 0.9495 |
| Rectal temperature (°C) | 38.79 | 38.84 | 38.84 | 0.09 | 0.8447 | 0.6041 | 0.6391 |
| Pulse (heart beats per minute) | 48.10 | 48.99 | 46.84 | 0.66 | 0.6419 | 0.3578 | 0.5742 |
| Respiration (no of breaths per minute) | 43.10 | 44.13 | 44.59 | 1.29 | 0.7131 | 0.5818 | 0.4297 |

[1]CTL, without any mineral supplementation; SeS, 3-gram Selemax® supplementation; ESeS, 3-gram Selemax® and vitamin E @ 6000 IU/day supplementation; [2]P value, probability, corresponding to null hypothesis; [3]SEM: standard error of mean; *P values of SeS and ESeS compared with CTL group by Dunnett test.

**Table 3:** Effect of Vitamin E and Selenium supplementation on semen traits of Holstein Frisian bulls during Summer

| | Treatment [1] | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|
| Variables | CTL | SeS | ESeS | SEM[3] | Treat | *CTL vs SeS | *CTL vs ESeS |
| Semen volume (ml) | 5.46 | 4.54 | 5.13 | 0.38 | 0.2727 | 0.1202 | 0.5710 |
| Semen concentration (million / ml) | 524.48 | 506.03 | 493.13 | 31.70 | 0.7866 | 0.6979 | 0.4963 |
| Motility (%) | 71.12 | 70.50 | 78.85 | 1.41 | 0.0026 | 0.7897 | 0.0042 |
| Progressive motility (%) | 23.02 | 21.43 | 19.64 | 1.64 | 0.3849 | 0.5050 | 0.1749 |
| Amplitude of lateral head displacement (micron meter) | 6.62 | 6.16 | 6.04 | 0.13 | 0.0191 | 0.0368 | 0.0075 |
| Straightness (%) | 83.51 | 85.24 | 84.08 | 0.48 | 0.0674 | 0.0264 | 0.4328 |
| DNA Integrity (%) | 95.10 | 95.25 | 95.72 | 0.49 | 0.6623 | 0.8421 | 0.4083 |
| Live to dead ratio | 57.62 | 60.11 | 61.82 | 1.59 | 0.2085 | 0.3024 | 0.0823 |
| Normal acrosomal ridge (%) | 45.68 | 44.44 | 45.26 | 0.76 | 0.5218 | 0.3222 | 0.7434 |
| Plasma membrane integrity (%) | 44.47 | 42.72 | 42.59 | 1.82 | 0.7239 | 0.5084 | 0.4777 |

[1]CTL, without any mineral supplementation; SeS, 3-gram Selemax® supplementation; ESeS, 3-gram Selemax® and vitamin E @ 6000 IU/day supplementation; [2]P value, probability, corresponding to null hypothesis; [3]SEM: standard error of mean; *P values of SeS and ESeS compared with CTL group by Dunnett test.

**Table 4:** Effect of Vitamin E and Selenium supplementation on blood metabolites and hormone of Holstein Frisian bulls during summer

| | Treatment [1] | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|
| Variables | CTL | SeS | ESeS | SEM[3] | Treat | *CTL vs SeS | *CTL vs ESeS |
| Glucose | 65.53 | 68.44 | 67.10 | 2.77 | 0.7626 | 0.4695 | 0.6946 |
| Blood urea nitrogen | 17.47 | 18.01 | 17.79 | 0.22 | 0.2462 | 0.1009 | 0.3266 |
| Testosterone | 11.93 | 13.69 | 14.66 | 0.42 | 0.0014 | 0.0104 | 0.0004 |

[1]CTL, without any mineral supplementation; SeS, 3-gram Selemax® supplementation; ESeS, 3-gram Selemax® and vitamin E @ 6000 IU/day supplementation; [2]P value, probability, corresponding to null hypothesis; [3]SEM: standard error of mean; *P values of SeS and ESeS compared with CTL group by Dunnett test.

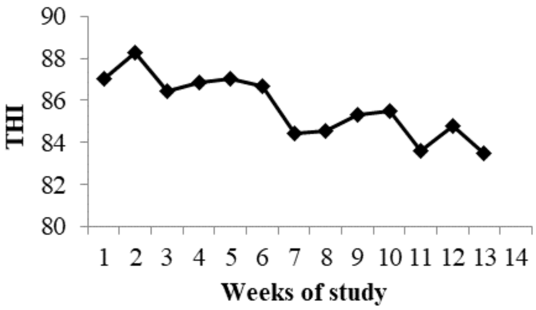


**Fig. 1:** Temperature-humidity index (THI) for 13 weeks of study period.

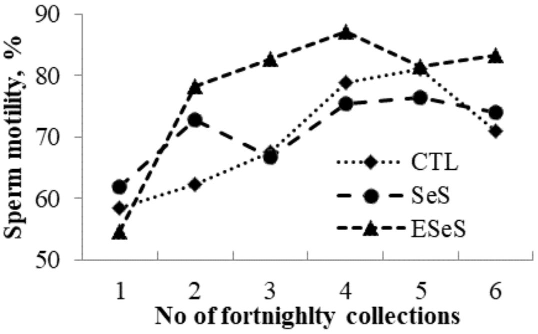


**Fig. 2:** Fortnightly sperm motility (%) of treatment groups. The groups were: CTL, without any mineral supplementation; SeS, 3-gram Selemax® supplementation; ESeS, 3-gram Selemax® and vitamin E @ 6000 IU/ day supplementation.

**Feed, water intake, and physiological measures:** Vit. E and Se supplementation did not influence feed and water intake (Table 2). Also, the rectal temperature, respiration and pulse rate were similar among the treatment groups (Table 2).

**Semen traits:** The results on semen traits are shown in Table 3. As conjoint, Vit. E and Se improved sperm motility. The ESeS group had significantly (P=0.0042) higher sperm motility (78.5%) compared to CTL (71.12%; Table 3). The improvement in sperm motility was evident from second collection onward (Fig. 2). Similarly, Vit. E and Se improved ALH values (Table 3). The SeS and ESeS groups had significantly lower ALH (6.16 and 6.04 µm, respectively) compared to CTL (6.62µm; Table 3). The SeS group had significantly higher straightness value (85.24%) compared to CTL (83.51%; Table 3). The conjoint effect of Vit. E and Se (ESeS group) showed an increasing trend (61.82; P=0.0823) on live to dead ratio compared to CTL (57.62; Table 3). The Vit. E and Se did not show any positive effect on semen volume, concentration, progressive motility, DNA integrity, normal acrosomal ridge, and plasma membrane integrity (Table 3).

**Blood metabolites and hormone:** Results of selected blood metabolites and testosterone analysis are shown in Table 4. Vit. E and Se supplementation did not influence blood glucose and BUN levels (Table 4). But, Vit. E and Se supplementation significantly improved testosterone levels in blood (P=0.0014). The testosterone was highest in ESeS (14.66 mg/dl) group followed by SeS (13.69) and CTL (11.93; Table 4).

## DISCUSSION

**Feed, water intake, and physiological measures:** The findings of present study indicating no effect of Vit. E and Se on DMI and water intake were in agreement to studies conducted in cattle where Vit. E and Se did not affect DMI in cows independently or in conjoint (Zhao *et al*., 2008). It could be postulated that the supplementation of Vit. E and Se did not improve the heat abatement abilities of the bulls nor those decreased the metabolic heat production. Bulls were under heat stressed throughout the study period and similar DMI showed that bulls of all the treatment groups experienced similar heat stress. Similarly, Tahmasbi *et al*. (2012) reported no effect of Vit. E and Se on DMI in Holstein dairy cows during hot weather. Regarding physiological measures, no effect of Vit. E and Se on RR, RT, and PR were in accordance to what has been reported in ewes and goats (QureShi *et al*., 2017; Zubair *et al*., 2017) and pigs (Liu *et al*., 2018). The similar physiological responses between the treatments reinforced our assumption that supplementation of Vit. E and Se did not influence the heat abatement capabilities of the bulls.

**Semen traits:** Sperm motility is negatively correlated with lipid peroxidation of spermatozoa membrane. Access of free radicals increase lipid peroxidation (Suleiman *et al*., 1996). Vit. E and Se decrease free radicals thereby improving sperm motility (Moslemi and Tavanbakhsh, 2011). The positive response of Vit. E and Se on sperm motility in our study could be explained due to their antioxidant properties of these nutrients (Domosławska *et al*., 2018) thereby decreasing oxidative stress during summer (Chauhan *et al*., 2014). The several previous studies in farm animals showed positive effects of Se and/or Vit. E on semen quality in goats (Shi *et al*., 2010), sheep (Liu *et al*., 2014) and bulls (Losano *et al*., 2018). Contrary to current study, no effect of Se and/or Vit. E in farm animals on reproductive performance could be attributed to the dose levels (Liu *et al*., 2014). Furthermore, it could be possible that animals were under sever heat stress and alone Se supplementation was not enough to show positive effects. However, Se in conjoint with Vit. E synergistically acted and improved the sperm motility as described by Domoslawska *et al*. (2015).

**Blood chemistry:** During heat stress, ruminants preferentially use glucose to minimize metabolic heat production (Baumgard and Rhoads, 2007). Preferential glucose utilization may increase gluconeogenesis from amino acids thereby increasing BUN. Similar glucose and BUN concentrations among groups indicated that bulls in all the treatment groups experienced same heat stress. The supplementation of Vit. E and Se did not reduce the physiological effects of heat stress. The current results were in agreement to the previous findings in cows (Tahmasbi *et al*., 2012; Khalifa *et al*.,2016) and buffaloes (Shinde *et al*., 2008). Glucose concentrations in our study were within the physiological boundaries. Likewise, no effect of Vit. E and Se was observed in previous studies (Tahmasbi *et al*., 2012; Alhidary *et al*., 2015) during summer. The increase in testosterone in response to Se (Shi *et al*., 2018) and Vit. E (Chen *et al*., 2005) have been reported previously. It was believed that these nutrients have role in promoting the testosterone synthesis by leydig cells (Shi *et al*., 2018).

The Vit. E and Se supplementation improved some important semen traits and testosterone levels in Holstein Friesian bulls during hot humid summer but did not improve the welfare of bulls.

**Acknowledgements:** The authors thank Mr. Imran (Lecturer, Department of Biochemistry, Ravi Campus, UVAS) for his assistance in chemical analysis, and Dr. Muhammad Naveed ul Haque (Assistant Professor, Department of Animal Nutrition, UVAS) for help in dose formulation of Vit. E and Se. The authors also acknowledge the contribution of the laboratory staff (Dr. Mirza Salman Saleem and Dr. Shazia Mansoor), technicians and bull handlers at semen production unit.

**Statement of animal rights:** The experimental procedures were approved by Ethical Review Committee, University of Veterinary and Animal Sciences, Lahore, Pakistan.

**Authors contribution:** MQS conceived and designed the study plan, analyzed the data, MAB collected the data and performed the analysis, JAB and AK contributed in manuscript write-up.

## REFERENCES

Agarwal A, Ikemoto I and Loughlin KR, 1994. Relationship of sperm parameters with levels of reactive oxygen species in semen specimens. J Urol 152:107-10.

Alhidary IA, Shini S, Al-Jassim RAM, *et al*., 2015. Effects of selenium and vitamin E on performance, physiological response, and selenium balance in heat-stressed sheep. J Anim Sci 93:576-88.

Baumgard LH and Rhoads RP, 2007. The effects of hyperthermia on nutrient partitioning. Proc Cornell Nutr Conf, Ithaca, NY pp:93-104.

Chauhan SS, Celi P, Leury BJ, *et al*., 2014. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J Anim Sci 92:3364-74.

Chen H, Liu J, Luo L, *et al*., 2005. Vitamin E, aging and Leydig cell steroidogenesis. Exp Gerontol 40:728-36.

Cocuzza M, Sikka SC, Athayde KS, *et al*., 2007. Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis, International Brazilian. J Urol 33:603-21.

Domosławska A, Zduńczyk S, Niżański W, *et al*., 2015. Effect of selenium and vitamin E supplementation on semen quality in dogs with lowered fertility. Bulletin of the Veterinary Institute in Pulawy 59:85-90.

Domosławska A, Zdunczyk S, Franczyk M, *et al*., 2018. Selenium and vitamin E supplementation enhances the antioxidant status of spermatozoa and improves semen quality in male dogs with lowered fertility. Andrologia 50:e3023.

Halliwell B and Whiteman M, 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean. Br J Pharmacol 142:231-55.

Khalifa HH, Safwat MA, El-Sysy MAI, *et al*., 2016. Effect of selenium and vitamin E supplementation as a nutritional treatment for some physiological and productive traits of Holstein dairy cows under Egyptian summer conditions. J Egyptian Acad Social Environ Dev 17:97-113.

Kelly CF and Bond TE, 1971. Bioclimatic factors and their measurements. In: A guide to environmental research in animals. Natl Acad Sci 7:92.

Khan MIR and Ijaz A, 2007. Assessing undiluted, diluted and frozen-thawed Nili-Ravi buffalo bull sperm by using standard semen assays. Ital J Anim Sci 6:784-7.

Khan MS, Rehman ZU, Khan MA, *et al*., 2008. Genetic resources and diversity in Pakistani cattle. Pak Vet J 28:95-102.

Koyuncu M and Yerlikaya H, 2007. Effect of selenium-vitamin E injections of ewes on reproduction and growth of their lambs. South African J Anim Sci 37:233-6.

Lewis SE and Aitken RJ, 2005. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 322:33-41.

Liu F, Celi P, Chauhan SS, *et al*., 2018. A short-term supranutritional vitamin E supplementation alleviated respiratory alkalosis but did not reduce oxidative stress in heat stressed pigs. Asian-Australas J Anim Sci 31:263.

Liu S, Masters D, Ferguson M, *et al*., 2014. Vitamin E status and reproduction in sheep: potential implications for Australian sheep production. Anim Prod Sci 54:694-714.

Losano JD, Angrimani DS, Dalmazzo A, *et al*., 2018. Effect of vitamin E and polyunsaturated fatty acids on cryopreserved sperm quality in Bos Taurus bulls under testicular heat stress. Anim Biotechnol 29:100-9.

Mirzad AN, Tada T, Ano H, *et al*., 2018. Seasonal changes in serum oxidative stress biomarkers in dairy and beef cows in a daytime grazing system. J Vet Med Sci 80:20-7.

Mortimer ST, Vander-Horst G and Mortimer D, 2015. The future of computer-aided sperm analysis. Asian J Androl 17:545.

Moslemi MK and Tavanbakhsh S, 2011. Selenium-vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. Int J Gen Med 4:99.

National Academies of Sciences, Engineering and Medicine (NASEM), 2016. Nutrient requirements of beef cattle: 8th revised ed. Washington, DC: The National Academies Press. https://doi.org/10.17226/19014.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle: 7th revised ed., 2001. Washington, DC: The National Academies Press. https://doi.org/10.17226/9825.

Qureshi MS, Akhtar S and Khan RU, 2017. The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. Appl Biol Chem 60:585-90.

Shinde PL, Dass RS, Garg AK, *et al*., 2008. Effect of vitamin E and selenium supplementation on growth, nutrient utilization and their balance in male buffalo calves. Anim Nutr Feed Techn 8:135-43.

Shi L, Song R, Yao X, *et al*., 2018. Effects of selenium on the proliferation, apoptosis and testosterone production of sheep Leydig cells in vitro. Theriogenology 15:24-32.

Shi L, Zhang CX, Yue W, *et al*., 2010. Shortterm effect of dietary selenium enriched yeast on semen parameters, antioxidant status and Se concentration in goat seminal plasma. Anim Feed Sci Tech 157:104-10.

Suleiman SA, Ali ME, Zaki ZMS, *et al*., 1996. Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl 17:530-7.

Tahmasbi AM, Kazemi M, Moheghi MM, *et al*., 2012. Effects of selenium and vitamin E and night or day feeding on performance of Holstein dairy cows during hot weather. J Cell Anim Biol 6:33-40.

Tartaglionea CM and Ritta MN, 2004. Prognostic value of spermatological parameters as predictors of in vitro fertility of frozen-thawed bull semen, Theriogenology 62:1245-52.

Tejada RJ, Mitchall JC, Norman A, *et al*., 1984. A test for practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil Steril 42:87-91.

Tvrdá E, Tušimová E, Zbyňovská K, *et al*., 2016. Protective effects of α-tocopherol on the activity and antioxidant profile of bovine spermatozoa subjected to ferrous ascorbate-induced oxidative stress. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 64:1245-55.

Velasquez-Pereira J, Chenoweth PJ, McDowell LR, *et al*., 1998. Reproductive effects of feeding gossypol and Vitamin E to bulls. J Anim Sci 76:2894-904.

Yousef MI, Abdallah GA and Kamel KI, 2003. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim Reprod Sci 76:99-111.

Zhao LL, De PY, Pu C, *et al*., 2008. Supplementation with Selenium and Vitamin E Improves Milk Fat Depression and Fatty Acid Composition in Dairy Cows Fed Fat Diet. Asian-Australas J Anim Sci 21:838-44.

Zubair M, Ahmad M, Saleemi MK, *et al*., 2018. Effects of vitamin E on hemato-biochemical constituents, kidney parenchyma and tissue accumulation in sodium arsenite exposed Teddy goats bucks. Pak Vet J 38:1-6.

Zubair M, Maqbool A, Saleemi MK, *et al*., 2017. Protective effects of vitamin E on sodium arsenite induced toxicity, testicular measurements and histopathological studies of testes in Teddy goat bucks. Andrologia, 49:e12699.