Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45** Issue **1 Year 2024** Page **456-462***

Libido and Semen Quality of Bali Bulls Supplemented with Micro-Nutrients of Zinc, Selenium, Vitamin A and Vitamin E

Abd. Latief Toleng[1*], Muhammad Yusuf[1], Sahiruddin[1] Masturi[1], Hasrin[2], Musdalifa Mansur[3]

[1]Laboratory of Animal Reproduction, Department of Animal Production Faculty of Animal Science Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10 Makassar, Indonesia
[2]Faculty of Vocational Studies, Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10 Makassar, Indonesia
[3]Animal Husbandry Study Program, Faculty of Science and Technology, Muhammadiyah Sidenreng Rappang University, Sidenreng Rappang, 91651, Indonesia

***Corresponding Author:** Abd. Latief Toleng*
**Email: latief@unhas.ac.id*

Abstract

Low quality and quantity of food intake of bulls in the tropics might adversely affect libido and semen quality through their effects on spermatogenesis process. The purpose of this study was to evaluate the effect of micro-minerals (Zn and Se) and vitamins (A and E) on the libido and semen quality of Bali bulls. A two Bali bulls, aged 4-5 years old with body weight of 300-350 kg were used in the study. The study was divided into two periods and each period was conducted for 8 weeks. At the first period (control), the experimental bulls were fed with natural grasses and concentrates (1% of body weight). Furthermore, at the second period (treatment period), they were fed diet similar to that used in the first period with an additional of a mineral mix (Zn and Se) and vitamin (A and E). Evaluation of libido and semen quality were carried out simultaneously twice a week. Libido was measured by calculating the time required for the bulls from approaching the teaser until they mounted for the first time and from approaching until they ejaculated, respectively. Semen was collected by using an artificial vagina. The volume, colour, pH, viscosity and mass movement were recorded. Furthermore, sperm concentration, motility, viability and abnormality were measured. Fresh semen was diluted by Andromed® and was preserved at the temperature 5ºC for 144 hrs. Motility, viability and abnormality were observed every 12 hrs. Data were analysed by using the paired student's t-test. Mean interval between the bulls approaching and mounting the teaser for the first time and between approaching and ejaculation after the supplementation of micro-nutrients were significantly shorter ($P<0.05$) in compared to those before the supplementation (2.68 vs. 6.52 and 5.09 vs. 10.06 minutes, respectively). The sperm viability after the supplementation of micro-nutrients was significantly higher in compared to that before supplementation. (82.25 vs. 87.50 %). Preservation of diluted bull semen at the temperature of 5ºC for 144 hrs showed that the sperm viability was significantly higher ($P<0.05$)

and sperm abnormality was significantly lower (P<0.05) after the supplementation of micro-nutrients in compared to those before the supplementation. It can be concluded that the supplementation of micro-nutrients in Bali bulls could increase libido, sperm viability and reduce the sperm abnormality.

Keywords: Spermatogenesis, micro-nutrient, libido, semen quality and Bali bull

INTRODUCTION

Low quality and quantity of food intake of bulls in the tropics might adversely affect libido and semen quality through their effects on spermatogenesis process. This problem might be related to the lower reproductive performance of cattle in these areas. Staub and Johnson (2018) have explained that Spermatogenesis is a long process in the tubules seminiferous to produce spermatozoa. Nutrients, especially micro nutrient affect spermatogenesis efficiency and finally reduce the libido and semen quality.

Hidiroglou and Knipfel (1984) have stated that low fertility in many species of domesticated animals was affected by the reduced of total RNA and protein content of spermatozoa which affected by Zinc. During spermatogenesis process, spermatid is very sensitive to the free radical oxidation. Some studies showed that Selenium (Se) can be used as an anti-oxidant to protect spermatozoa. Beckett and Arthur (2005) have shown that lower Se level in selenoprotein caused oxidative stress of the spermatozoa and reduce fertility. The availability of Se in sufficient quantities in the diet ensures the proper functioning of the immune and reproduction systems (Mehdi and Dufrasne, 2016).

Cheah and Yang (2011) have reviewed the important roles of vitamins on the spermatogenesis proses. Vit. B12 involves in RNA and DNA synthesis and promotes healthy growth of seminiferous tubule, Vit. B9 promotes healthy sperm and seminiferous tubule development, Vit. A have a role of spermatogonia differentiation and spermatid adhesion regulation, Vit. E promotes healthy reproductive organs development and prevents sperm cell membrane from lipid peroxidation, defends sperm from ROS-related events, and Vit. C protect sperm from oxidative stress.

We have reported that the supplementation of *Moringa* leaves could increase plasma testosterone concentrations, libido, and sperm motility of Bali bulls (Syarifuddin, et al., 2017). The moringa leaves are rich in nutritive values. The levels of crude protein content is 30.29%, fat is 6.50%, ash is 7.64%, calcium is 3.65%, phosphorus is 0.3%, neutral detergent fibre is 11.4%, acid detergent fibre is 8.49%, acid detergent lignin is 1.80%, acid detergent cellulose is 4.01%, Zn is 31.03 mg/kg (Moyo et al., 2011), saponin is 80 g/kg (Ferreira et al., 2008), phenol is 8 µg/mL, flavonoids is 27 ug/mL (Rajanandh and Kavitha, 2010), and alkaloid is 0.07% (Madukwe et al., 2013), ferulic acid is 46.8 mg/g, and chlorogenic acid is 18.0 mg/g (Fitri et al., 2015). In addition, Krisnadi (2014) has reviewed that Vit. A in moringa leaves has 10 times in compared to carrot, Vit. E has 4 times in compared to corn oil and Zinc has 6 times in compared to almond.

Although it was detected that moringa leaf significantly improved libido and semen quality of the bulls, however, it was not clarified yet whether this effect was related to the high level of micro nutrients in the moringa leaf. Therefore the aims of this study were to evaluate the effect of micro-minerals (Zn and Se) and vitamins (A and E) on the libido and semen quality of Bali bulls.

MATERIALS AND METHODS

This study was conducted in a tropical area of Indonesia using two Bali bulls, aged 4-5 years old with a body weight of 300 - 350 kg and they were kept in an individual barn. The study was divided into two periods of experiments and each period was conducted for eight weeks. The first two weeks of each period were conducted as the adaptation phase. At the first period, the experimental bulls were fed with natural grasses and rice straw ad lib. They were also fed with concentrates (1% of body weight) (control). Furthermore, at the second period, they were fed diet similar to that used in the first period with an additional of a mineral mix (Zn 200 mg/bull/day and Se 20 mg/bull/day) as well as vitamin A (37,500 IU/bull/day) and Vitamin E (700 IU/bull/day). Measurements of libido and semen quality were carried out simultaneously twice a week. Libido was measured by calculating the time required for the bulls from approaching the teaser until they mounted for the first time and until ejaculated, respectively. Before semen collection, the false mounting was conducted twice.

Semen was collected by using an artificial vagina. The collected semen was handled according to the procedures of Susilawati (2011). The volume, colour, pH, and, viscosity, as well as a mass movement, were recorded. Evaluation of semen quality at each collection was conducted soon after collection. Sperm concentration was evaluated using Photometer SDM 6. Computer Assisted Semen Analyser (CASA) with Sperm Vision™ Version 3.7.5 software was used to determine the motility of the sperms, while viability and abnormality were assessed in a trinocular microscope. Fresh semen was diluted by Andromed® and the diluted semen was preserved at the temperature of 5ºC for 6 days. Motility, viability and abnormality were observed every 12 hrs.

Statistical Analysis

Data obtained in the study were tabulated and calculated using Excel program for Windows. Descriptive statistics in the Excel program was used to obtain the mean and standard error (SE) of data. Differences in sperm concentration, motility, viability and abnormality were analysed by using the paired student's t-test with SPSS® Version 22 software.

RESULTS AND DISCUSSION

Libido of Bali Bulls before and after Supplemented with Micro-Nutrients

The interval between the bulls approaching and mounting the teaser for the first time and between approaching and ejaculation are shown in Table 1.

Table 1: The interval between the bulls approaching and mounting the teaser for the first time and between approaching and ejaculation before and after the supplementation of micro nutrients

Parameter	Micro-nutrient supplementation	
	Before	After
Intervals between the bull approaching and Mounting the teaser for the first time (min.)	6.52±2.02[a]	2.68±2.52[b]
Intervals between the bull approaching and Ejaculation (min.)	10.06±1.02[a]	5.09±2.68[b]

[ab] Means in the same row with different superscripts differ significantly (P<0.05).

Mean interval between the bulls approaching and mounting the teaser for the first time and between approaching and ejaculation after the supplementation of micro-nutrients were significantly shorter (P<0.05) in compared to those before the supplementation (2.68 vs. 6.52 and 5.09 vs. 10.06 minutes, respectively). These data showed that the supplementation of micro-nutrients could increase libido of Bali bulls. This result was supported by Supriyanto (2018), who stated that supplementation of micro-nutrient could increase libido of the bulls. He found also that high libido was also related to the high intensity of erection.

The result of this study is supported by Supriyanto (2018) stated that supplementation of micro-nutrients would be able to increase the libido of the bulls. High quality of erection of the bulls resulted high level of libido. Supplementation of vitamin A and E, and Zn in an herbal mixed with moringa leaf increased libido related to the high plasma testosterone. High testosterone induce protein synthesis in the epididymis cells and spermatogenesis process in the seminiferous tubules and induce the erection (Shalaby, et.al, 2020). Zn also affects testosterone synthesis process. This mineral induce Leydig cells in the testes to produce testosterone for increasing libido (Roy et al., 2013).

Shorter interval between the bulls approaching and mounting the teaser for the first time found in the present study and it was similar to those reported by Sam et al. (2017) and Abdullah et al. (2007). In goats, Abdullah et al. (2007) reported that supplementation of fish mill and 2% mineral in feed could shorter the interval between approaching the female and mounting for the first time. Cheah and Yang (2011) have reported that the supplementation of mineral and vitamin in fed affect sertoli and leydiq cells and then increase the libido of the bulls. Similar results of high libido for bulls supplemented with micro-nutrients found in this study were similar to those reported in bulls supplemented with moringa leaf (Syarifuddin et al., 2017). This result indicate that the high levels of Zn, Se, vitamin A and vitamin E have an important role in increasing libido of the Bali bulls.

Quality of Bali Bulls Semen before and after Supplemented with Micro-Nutrients

a. Fresh semen quality

Macroscopic and microscopic evaluation of Bali bulls fresh semen before and after micro-nutrients supplementation shown in Table 2.

Table 2: Macroscopic and microscopic evaluation of Bali bulls fresh semen before and after micro-nutrients supplementation

Parameter	Micro-nutrient supplementation	
	Before	After
Macroscopic evaluation		
Volume (ml)	5.4±0.86	5.32±0.47
pH	6.5±0.0	6.50±0.20
Colour	yellowish	Yellowish
Viscosity	Medium	High
Microscopic evaluation		
Motility (%)	87.18±3.55	85.35±3.57
Viability (%)	90.00±0.82	90.25±4.03
Abnormality (%)	11.00±2.45	9.50 ± 3.11
Concentration (x 10^6/ml)	1,204±438.27	1,506±398.65

Volume, pH and colour of the bull semen were not significantly different (P>0.05) between the two treatments. Mean volume per ejaculation, pH and colour of semen in both groups were 5.3-5.4 ml, 6.5 and yellowish, respectively. Viscosity of the semen was higher in the treated bull in comparison to that in control group. These results were similar to those reviewed by Saili et al. (2023). They reported that the volume of Bali bulls semen was 5.33 – 7.2 ml, pH 6.27 – 7.0 and colour white to yellowish. Similar results were also shown by Susilawati (2011).

Table 2 shows that motility, viability and abnormality of Bali bulls before and after the supplementation of micro-nutrients were similar (P>0.05). The normal motility, viability and abnormality of semen reported in this study was supported by Saili et al. (2023), Susilawati (2011), Prastika et al. (2018) and Blegur et al. (2020).

b. Diluted semen quality

Diluted Bali bulls semen quality before and after the supplementation of micro-nutrients shown in Table 3.

Tabel 3: Diluted Bali bulls semen quality before and after the supplementation of micro-nutrients shown.

Semen quality	Supplementation of micro-nutrients	
	Before	After
Motility (%)	84.18±2.50	84.73±3.020
Viability (%)	82.25±2.50[a]	87.50±2.08[b]
Abnormality (%)	13.75±2.21	10.5±3.10

[ab] Means in the same row with different superscripts differ significantly (P<0.05).

Motility and abnormality of spermatozoa in the diluted semen were not significantly different (P>0.05) between before and after the supplementation of micro-nutrients. However, the sperm viability after the supplementation of micro-nutrients was significantly higher in compared to that before supplementation. (82.25 vs. 87.50 %). The increase of viability of spermatozoa after the supplementation of micro-nutrients found in this study was supported by Geary et al. (2021). They reported that trace mineral supplemented versus no mineral supplementation had prolonged viability and ability to withstand oxidation stress.

c. Sperm quality during preservation

Sperms motility during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A and Vit. E) are shown in Figure 1.

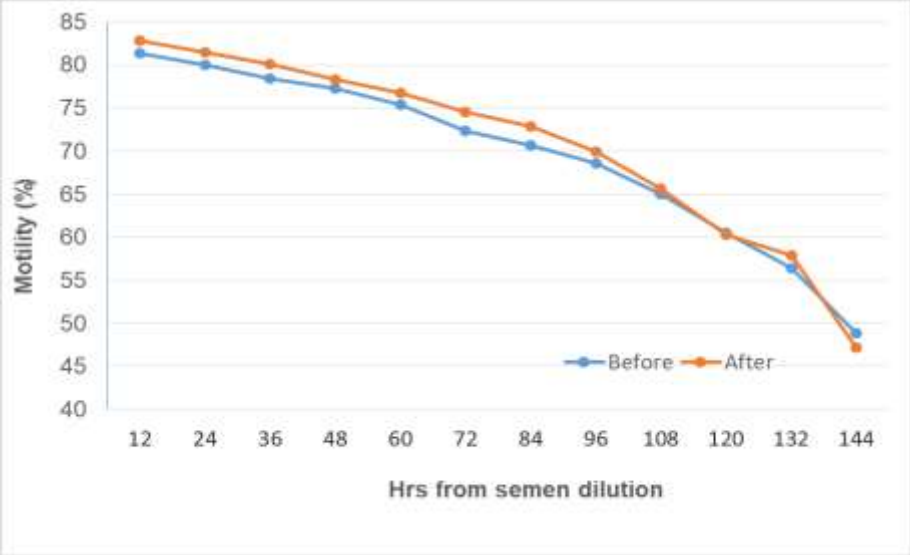

Figure 1: Sperm quality during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E)

Sperms motility before and after supplemented with micro-nutrient were gradually decreased during preservation at 5ºC and reached the lower level (<50 %) at 144 hrs after the beginning of preservation. There was no significant different (P>0.05) between the sperm motility before and after the supplementation of micro-nutrients. Sperm viability during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E) are shown in Figure 2.

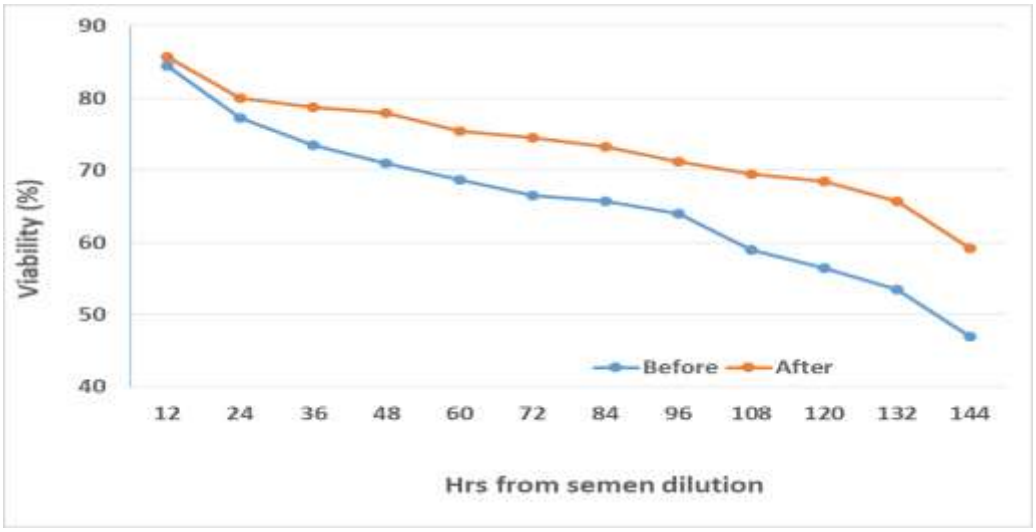

Figure 2: Sperm viability during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E).

Viability of diluted semen preserved at 5ºC for 144 hrs after the supplementation of micro-nutrients was significantly higher (P<0.05) in compared to those before the supplementation. Viability of the sperms in the two groups were gradually decreased during the preservation. However, the sperm viability before the supplementation reached the lower level (<50 %) faster in compared to those after the presentation. Sperm abnormality during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E) are shown in Figure 3.

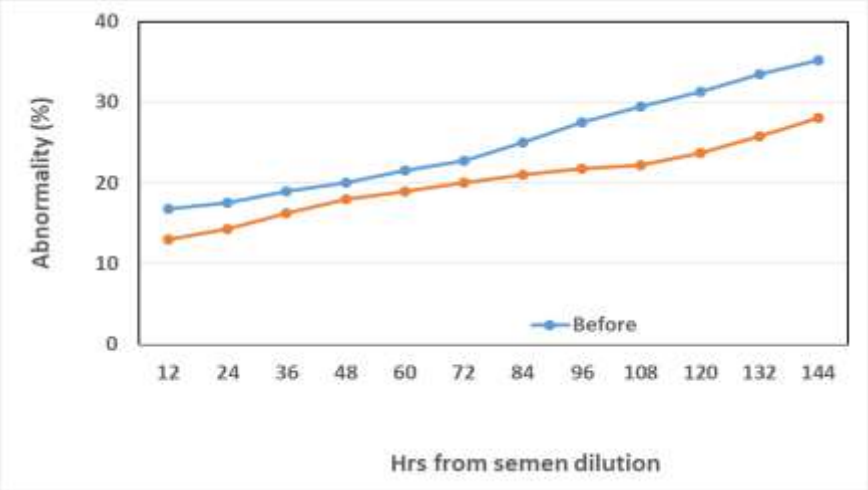

Figure 3: Sperm abnormality during preservation at 5ºC for 144 hrs before and after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E)

Sperm abnormality during preservation at 5ºC for 144 hrs after the bulls supplemented with micro-nutrients (Se, Zn, Vit. A dan Vit. E) was significantly lower ($P<0.05$) in compared to that before the supplementation. These data showed that the preservation of diluted bull semen at the temperature of 5ºC for 144 hrs showed that the sperm viability was significantly higher ($P<0.05$) and sperm abnormality was significantly lower ($P<0.05$) after the supplementation of micro-nutrients in compared to those before the supplementation. However, the sperm motility were remain similar between the two groups of micro-nutrients supplementation.
The similar response sperm mortality in the two groups of treatments detected in this study was in contrast to those reported by Hindrawati et al. (2020) who showed that supplementation of Zn in Bos Indicus bulls induced a high level of motility in compared to control group. Kurnia et al. (2020) reported that Se in micronutrients is an anti-oxidant and Zn involve in spermatozoa development that increase plasma androgen level which cause the sperm motility. The discrepancy of these results with the results of this study was not clarified yet. It might be due to enough levels of Zn and Se in the basal diet used in the present study.
Preservation of semen in 5ºC for 144 hrs were gradually reduced sperm viability before and after the supplementation of micro-nutrients. However, viability of sperm in micro-nutrients supplemented bull was higher in compared to those before the supplementation. This result indicated that micro-nutrients could reduce the negative effects of free radicals and oxidative stress (Kurnia et al., 2020). Supplementation of micro-nutrients of bulls could reduce sperm abnormality spermatozoa during the semen preservation in 5ºC for 144 hrs. This results indicated that anti-oxidant in micro-nutrients could protect spermatozoa from free radical and oxidative stress (Hindrawati et al., 2020).

CONCLUSION

It can concluded that the supplementation of micro-nutrient such as Selenium (Se), Zinc (Zn), Vitamin A and Vitamin E could increase libido and viability of Bali bull spermatozoa.

ACKNOWLEDGMENT

The authors are thank to Lembaga Penelitian dan Pengabdian kepada Masyarakat; (LPPM) Hasanuddin University (Institute for Research and Community Service; IRCS) for funding this study based on contract No. 00323/UN4.22//PT.01.03/2023, January 25, 2023 for research scheme of Collaborative Fundamental Research (Penelitian Fundamental Kolaborasi; PFK 2023).

AUTHOR'S CONTRIBUTION

ALT and MY contributed in designing, collecting and analysing data, as well as drafting the manuscript. SR, MT, HR and MM contributed in collecting data, adjusting the data and reading the manuscript.

REFERENCES

1. Abdullah M. Kusmartono, Suyadi, Soebarinoto, Winugroho. 2007. Pengaruh Pemberian Tepung Ikan Lokal dan Import terhadap Pertambahan Bobot Badan, Tingkah Laku Sexual dan Produksi Susu Kambing Kacang. Jurnal Animal Production, 9(3): 135-144.
2. Blegur J, Nalley WM, Hine TM. 2020. Pengaruh penambahan virgin coconut oil dalam pengencer tris kuning telur terhadap kualitas spermatozoa sapi Bali selama preservasi. Jurnal Nukleus Peternakan, 7(2): 130-138.
3. Cheah Y, Yang W. 2011. Functions of essential nutrition for high quality spermatogenesis. Advances in Bioscience and Biotechnology, 2011, 2, 182-197 doi:10.4236/abb.2011. 24029 Published Online August 2011 (http://www.SciRP.org/journal/abb/).
4. Beckett GJ, Arthur JR. (2005) Selenium and endocrine systems. Journal of Endocrinology, 84, 455-465.
5. Ferreira PMP, Farias DF, Oliveira JTA, Carvalho AFU. 2008. Moringa oleifera: bioactive compounds and nutritional potential. Review. Rev. Nutr. 21 (4) https://doi.org/10.1590/S1415-52732008000400007
6. Geary, TW, Waterman RC, VanEmon ML, Ratzburg CR, Lake S, Eik BA, Armstrong DR, Zezeski AL, Heldt JS. 2021. Effect of supplemental trace minerals on standard and novel measures of bull fertility. Theriogenology, 172, p307-314.
7. Hidiroglou M, Knipfel JE. 1984. Zinc in mammalian sperm: A review. Journal of Dairy Science, 67, 1147-1156.
8. Hindrawati SG, Ciptadi, Chuzaemi S. 2020. Kajian suplementasi zinc organik terhadap kualitas semen pejantan sapi Bos Indicus. Journal of Tropical Animal Production, 21(2): 237-245.
9. Fitri A, Toharmat T, Astuti DA, Tamura H. 2015. The potential use of secondary metabolites in Moringa oleifera as an antioxidant source. Med. Pet. 38:169-175. https://doi.org/10.5398/medpet.2015.38.3.169
10. Krisnadi DA. 2014. Kelor Super Nutrisi. Pusat Informasi dan Pengembangan Tanaman Kelor Indonesia. Lembaga Swadaya Masyarakat – Media Peduli Lingkungan (LSM Mapeling).
11. Kurnia A, Soeparna I, Arifiantini, Hidayat R. 2020. Performa sapi Simental yang diberi imbuhan selenium dan zinc dalam pakan. Acta Veterinaria Indonesia, 8(1): 24-31.
12. Madukwe EU, Ezeugwu JO, Eme PE. 2013. Nutrient composition and sensory evaluation of dry Moringa oleifera aqueous extract. Int. J. Sci. Basic Appl. Res. 13: 100-102.
13. Mehdi Y, Dufrasne I. 2016. Selenium in cattle: A review. Molecules. 21(4), 545; https://doi.org/10.3390/molecules21040545
14. Moyo B, Masika PJ, Hugo A, Muchenje V. 2011. Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. African J. Biotech., 10 (60): 12925-12933.
15. Prastika, Z. S. Susilowati., B. Agustono., E. Safitri., F. Fikri, dan R. A. Prastiya. 2018. Motilitas dan viabilitas spermatozoa sapi Rambon di desa Kemiren banyuwangi. Jurnal Medik Veteriner, 1(2): 38-42.
16. Rajanandh MG, Kavitha J. 2010. Quantitative estimation of β-sitosterol, total phenolic and flavonoid compound in the leaves of Moringa oleifera. Int. J. Pharmtech. Res. 2:1409-1414.
17. Roy, B., R.P.S. Baghel, T.K. Mohanty and G. Mondal. 2013. Zinc and Male Reproduction in Domestic Animals: A Review. Indian Journal of Animal Nutrition 30(4): 339-350.
18. Saili T, Nafiu LO, Pagala MA, Bain A, Aku AS, Rahadi S, Rusdin M, Lopulalan F. 2023. Sperm quality of Bali bull following sexing and freezing using different cryoprotectants. In IOP Conference Series: Earth and Environmental Science 2023 Sep 1 (Vol. 1241, No. 1, p. 012137). IOP Publishing.
19. Sam, A, F., E. Pudjihastuti, M. J. Hendrik, L. Ngangi dan I.G.P. N. Raka,. 2017. Penampilan Tingkah Laku Seksual Sapi Pejantan Limousin dan Madura di Balai Inseminasi Buatan Lembang. Jurnal Zootek. 37 (2) : 276-285.
20. Shalaby AM, Alabiad MA, El Shaer DF. 2020. Resveratrol ameliorates the seminiferous tubules damages induced by finasteride in adult male rats. Microscopy and Microanalysis. 1;26(6):1176-86.
21. Supriyanto, S. 2018. Pengaruh Tingkah Laku Seksual Terhadap Produksi Semen Pada Sapi Madura. Doctoral Dissertation, Universitas Brawijaya.
22. Staub and L. Johnson (2018) Review: Spermatogenesis in the bull. Animal (The Animal Consortium 2018): 1-9.
23. Susilawati T. 2011. Spermatologi (In Indonesian). Universitas Brawijaya Press (UB Press), Malang.
24. Syarifuddin, N. A. 2021. Daun Kelor Meningkatkan Libido dan Kualitas Sperma Sapi Bali. Bintang Pustaka Madani : Yogyakarta, 1-2.