The effect of *Moringa oleifera* leaves on the quality of Bali bulls semen

The effect of *Moringa oleifera* leaves on the quality of Bali bulls semen

M A Jamili[1], A L Toleng[2], M Yusuf[2], Sahiruddin[2], Masturi[2] and Hasrin[2]

[1]Department of Animal Husbandry, Faculty of Science and Technology Universitas Islam Negeri Alauddin Makassar, Jl. H.M. Yasin Limpo No. 36 Samata Gowa (92113) Indonesia
[2]Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan KM.10 Makassar (90245), Indonesia

E-mail: arsan.jamili@uin-alauddin.ac.id

Abstract. This study aimed to investigate the effects of *Moringa oleifera* leaf (MOL) as a feed supplement on the semen quality of Bali bulls. The study was divided into two periods of 8 weeks (before and after) the supplementation of MOL. Four Bali bulls, weighing 250-350 kg, aged 3 to 4 years old were used in this study. At the second period (treatment period), the bulls were supplemented with dried MOL 0.1% of body weight. Semen was collected from the bulls once a week using an artificial vagina. The parameters in this study were macroscopic and microscopic evaluation of semen. Macroscopically evaluation of semen consisted of volume, pH, color, and viscosity. Microscopically evaluation of semen included mass movement, total sperms motility, and semen concentration. Microscopic evaluation of semen was conducted using Computer-Assisted Sperm Analysis (CASA). Furthermore, the data obtained were analyzed using SPSS with a Paired T-Test Sample design model. The results of this study showed that the quality of Bali bulls semen that supplemented with MOL had significantly ($P<0.01$) higher than the control group, both macroscopic and microscopic evaluation. Supplementation of MOL enhanced volume, color, viscosity, mass movement, sperm motility, sperm concentration of Bali bulls' semen. In a conclusion, supplementation of MOL in the feeding of Bali bulls improved the semen quality.

1. Introduction

The success of the reproductive process in cattle is largely determined by the condition of the male and female during mating. One of the most important parameters in male cattle is the quality of semen produced to fertilize eggs from female cattle [1]. Low quality of semen had a negative impact on reducing the conception rate (C/R) [2] and the calving rate of calves.

In the process of cattle reproduction, it can run normally if the nutritional needs of good feed for growth and reproduction can be fulfilled optimally. In male cattle, the completeness of nutrients in male feed can maintain the quality of the sperms produced and the function of the endocrine [3]. One type of feed that can be used as a good source of nutrition for male reproduction is Moringa leaves. Low intake of dietary nutrients can also result in a decrease in the number of pre-ejaculate sperms and loss of libido. In young animals, feed with low nutrition causes a delay in puberty.

Moringa leaves are a great alternative animal feed, especially during the dry season when animal feed is very limited [4]. Production of biomass for feed reaches 4.2-8.2 tons/Ha and is an alternative that

can substitute commercial livestock rations [5] and good for use as a feed supplement [6]. Moringa leaves are rich in nutrients. Crude protein content is 30.29%, fat 6.50%, ash 7.64%, calcium 3.65%, phosphorus 0.3%, neutral detergent 11.4%, acid detergent 8.49%, acid detergent lignin 1.80%, acid detergent cellulose 4.01%, Zn 31.03 mg/kg [7], saponin 80 g/kg [8], phenol 8 µg/mL, a- flavonoids 27 µg/mL [9], and alkaloid 0.07% [10] ferulic acid 46.8 mg/g, dan chlorogenic acid 18.0 mg/g [6]. Moringa leaves also contain tannins (21g/kg DM) and phytate (21g/kg DM) which are so small that they can be ignored as anti-nutrients for ruminants. Moringa leaves do not contain trypsin and inhibitor amylase, lectins, cyanogenic glycosides, dan glucosinolates [8].

Many studies have been conducted regarding the use of Moringa leaves as feed. However, research has looked at the link between Moringa leaves and libido and sperm quality, especially for bulls, it is still very limited. In rabbits, Abu et al. [11] showed that the Moringa leaf meal did not hurt the quality of sperms in the epididymis. These results indicate that Moringa leaves can be used as feed for rabbits. On goats Red Sakoto, Raji & Njidda [12] showed that supplementation of Moringa leaf feed 50% can increase sperm count. The latest publication on cows that the supplementation of *Moringa oleifera* Leaf (MOL) powder block could increase the conception rate of Bali cows [13]. Moreover, milk production and protein concentration in the milk of dairy cattle could be increased by supplementation of MOL powder block [14]. These results also indicate that Moringa can be used in ruminants, especially for cattle. Therefore, this study was conducted to examine the effect of supplementing Moringa leaves on the semen quality of Bali bulls.

2. Materials and methods

Four Bali Bulls, 250-350 kg of bodyweight 3-4 years old, were used in this study. They were kept individually in animal house. All animals were fed with elephant grass with an additional concentrate that consisted of rice bran, soybean meal, molasses, and mineral mix. The study was divided into 2 periods, at the first period (control period), the animals were not supplemented with dried *Moringa oleifera* leaf (MOL). At the second period (treatment period), they were supplemented with MOL of 250 g/head/day. Each period lasted for 8 weeks.

Semen collection was conducted twice a week by using an artificial vagina as described by Hafez et al. [15]. To increase the libido of Bali bulls, a teaser cow was used during semen collection. The collected semen was evaluated both macroscopic and microscopic. The macroscopic evaluation was done to examine the physical quality of semen. The parameters measured in this evaluation were volume, pH, color, and viscosity. Only good physical quality semen was proceeded to the microscopic evaluation by using Computer-Assisted Sperm Analysis (CASA). Parameters measured in this microscopic evaluation were mass and individual movements of the sperms, total sperm motility, and concentration of sperms.

The different means of sperm motility and concentration between control and treatment periods were analyzed using paired Student t-test [16].

3. Results and discussion

3.1. Effect of MOL on the macroscopic quality of semen

The macroscopic quality of Bali bulls semen before and after the supplementation of MOL was shown in table 1. The volume of semen, consistency, and semen color of the bulls before supplementing MOL were significantly ($P < 0.01$) lower than those bulls after supplementing MOL, while the pH and mass movement of the sperms did not showing any significant differences ($P > 0.05$). Before supplementing MOL, the semen volume was 2.49 mL, medium consistency, and milky white color. After supplementing the MOL, the average volume of semen increased to 4.24 mL, the consistency became slightly thick and creamy. However, pH and mass movement did not change before and after supplementation.

Table 1. Macroscopic evaluation of semen before and after supplementation of MOL.

Parameters	Supplementation of MOL	
	Before	After
Volume (mL)	2.49± 0.88[a]	4.24 ± 0.4[b]
pH	6.99 ± 0.02	6.99 ± 0.03
Consistency	Moderate (2.4 ± 0.64)[a]	Slightly Thick (3.4 ± 0.15)[b]
Color	White Milk (2.19 ± 0.12)[a]	Creamy (3.21 ± 0.21)[b]
Mass movement	++	++

[ab]Superscripts at different rows differed significantly (P<0.01).

Previous study of Ratnawati et al. [2] using traditional herbal supplementation (chicken eggs, honey, key meeting, and vitamin E) showed that the volume of semen was 5.6 mL, with a medium-viscous consistency, and cream-white milk color, mass movement "++" and pH 6.5–6.9. In general, these results did not differ significantly in comparison obtained in the present study using MOL. However, the volume obtained in this study was still at normal levels. This is in line with the study of Partodihardjo [17] stated that the average volume of bull ejaculation is 4-5 mL. Another study [18], stated that the average volume of bulls' semen is 5-8 mL. Likewise, Bardan, et al. [19] stated that the volume of Bali bulls is 3.8 mL/ejaculation. The present study suggests that after supplementing MOL the volume of semen in Bali bulls increased significantly.

Several factors can affect the volume of semen per ejaculate. As explained by Partodihardjo [20] that the factors that influence the volume of semen in cattle are the age of the bull, the breed of the animal, the size and weight of the animal, the frequency of semen collection, and the most important is the nutritional content of the feeding. The different semen consistency before and after supplementing MOL was related to the color of the semen. According to Ramsiyati [21], the thick semen will be milky white or cream.

The nutritional intake of the bulls had an impact on semen quality. MOL has good nutrition because it contains protein, minerals, vitamins, and other nutritional components that can improve the ejaculation volume. According to Dethan et al. [22] that feed with high protein content will produce better semen characteristics including the volume produced. One of the nutritional content of MOL which is also able to increase the volume of semen is Zn. A study by Kumar et al. [23], proved that organic and inorganic Zn can improve the quality and quantity of semen.

The pH of Bali bulls semen obtained in this study was at normal levels, indicated that the semen could be further processed. Wahyuningsih et al. [24] stated that the pH range of semen that can be processed further is 6.58 - 7.00. A similar study [25] stated that the pH of the Bali bulls is 6.4 - 7.8, and another study [26], stated that the normal semen pH is 6.2 - 7.5. The difference in the pH values among the bulls is influenced by several factors. the pH of the semen tends to be alkaline might be caused by more fluids produced by the accessories gland, whereas high semen pH is caused by a lot of dead sperms [27].

The consistency and color of the semen collected from the Bali bulls that supplemented with MOL have a thick consistency with a milky white color, which is most probably caused by high nutrients in the diet. Dewi et al. [1] stated that the factor that influences semen viscosity is the quality of the feeding. The better quality of the feeding will produce thick semen and vice versa. MOL supplements in the present study have been shown to improve sperms consistency.

3.2. Effect of MOL on the microscopic quality of semen

The average mass movement of the sperms obtained in this study was 2.8 (++/+++) and 2.4 (++) for those Bali bulls supplemented with and without MOL, respectively. The importance of mass movement evaluation in semen is intended only to determine for further processing of semen. Toelihere [19] stated that a minimum mass movement that can be processed is ++. This indicated that the semen collected in the present study can be further processed.

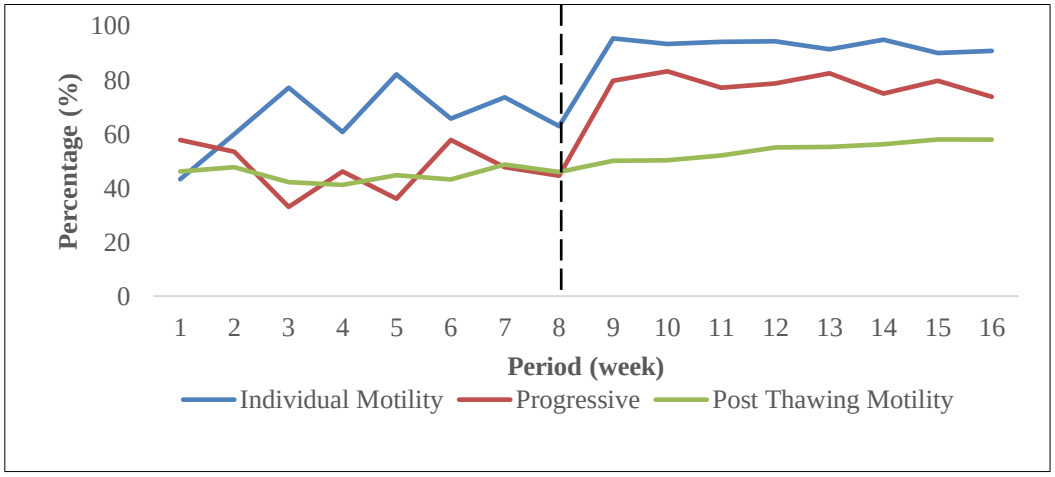

Figure 1. Microscopic evaluation of Bali bulls semen before and after supplementing MOL.

3.3. Individual motility of Bali bulls sperms

Figure 1 shows the motility of sperms before and after supplemented with MOL. The average individual motility of sperms before supplementing MOL was 70.11 ± 8.80 %, significantly (P<0.01) lower than the average individual motility of sperms after supplementing MOL (92.99% ± 2.04 %). The average individual motility of sperms obtained in this study is in line stated by Sarastina et al. [28] that the average motility of all cattle breeds is above 80%. Study by Ratnawati et al. [2] using traditional herbal medicine was reported 88.7 ± 5.5 %.

The existence of a significant difference proves that the nutritional content of MOL is good for sperms motility. According to Yunsang Cheah and Wanxi Yang [29], explained that several nutrients that affect sperms motility include zinc, selenium, vitamins C and E, calcium, and nickel. Meanwhile, Moyo, et al. [7] and Kathryn [30] stated that Moringa leaves contain all these nutritional elements.

3.4. Progressive motility of Bali bulls sperms

The progressive motility of Bali bulls sperms after supplementation of MOL showed significantly (P<0.01) higher than those before supplementation of MOL (78.74 % vs. 47.05 %) (Figure 1). Sarastina et al. [28] stated that the minimum standard of good progressive motility is 60%. This study confirms that progressive motility of the bulls after supplementing with MOL exceeds the standard rate. The factor may influence this progressive motility is the content of L-carnitine in the MOL.

Study of Zhou et al. [31] showed that supplementation with carnitine improved sperms quality or quantity in the testicles of mice exposed to physical damage, such as heat and X-ray radiation. Mature spermatozoa are protected by carnitine by absorbing excess acetyl-CoA from mitochondria and storing it in the form of L-acetyl-carnitine. In addition, it also inhibits protein oxidation and oxidative lactate damage by removing excess intracellular toxic acetyl-CoA. L-arnithine (LC) and its L-acetyl-carnitine (LAC) derivative have been reported to improve male infertility by increasing sperm progression. In addition, vitamins C and E in the MOL might be also work as anti-oxidants.

3.5. Post thawing motility (PTM) of Bali bulls sperms

The mean rate of decrease in the percentage of sperms after thawing for the bulls' semen before and after supplementation of MOL differ significantly (P<0.01) (38.32% vs. 54.34%). It can be seen that the sperms of the bulls supplemented with MOL was 54.34%; means that percentage of post-thawing motility is still suitable for Artificial Insemination. Toelihere [18] stated that post-thawing motility (PTM) of frozen semen that is suitable for Artificial Insemination is around 40%.

The high rate of post-thawing spermatozoa motility of the bulls supplementing with MOL is caused by the content of Moringa in the form of Vitamins C and E. This is presumably because, with vitamin C, the rate of fructolysis is optimized so that energy needs for motility and survival can be met. Also, it

is thought that vitamins C and E can bind to oxygen radicals present in cells, to prevent the formation of lipid peroxidation which can inhibit glycolysis and motility. Chinoy et al. [32] reported that administration of vitamin C in combination with calcium was found to increase Na^+ and K^+ levels as well as ATPase and succinic dehydrogenase activity, increasing spermatozoa energy metabolism.

Hydrosinonenal (HNE) is a lipid peroxidation that can inhibit glycolysis and motility of spermatozoa. Inhibition of motility by HNE is thought to be related to inhibition of glycolysis and oxidation of sulfhydryl groups (-SH) of spermatozoa tail microtubule protein. Vitamin C is an antioxidant that can bind radical compounds so that it can prevent the formation of lipid peroxidation [33].

The decrease in the percentage of spermatozoa motility after freezing in the control group was probably caused by the fewer sperms that had sufficient energy reserves to be used for movement because spermatozoa that have experienced cold stress can experience membrane destabilization. Aslam et al. [34] stated that membrane destabilization will increase membrane permeability to ions, including calcium ions resulting in an increase in calcium ions in the cytosol followed by an increase in calcium ions in the mitochondria. Increasing the concentration of calcium ions in the mitochondria will reduce the synthesis of ATP, as a result, the energy reserves that can be used for spermatozoa motility will decrease [35]. Besides, freezing causes a decrease in spermatozoa motility due to the presence of lactic acid, a residual cell metabolism that causes the medium to become increasingly acidic. This condition can be toxic to spermatozoa which eventually causes the death of spermatozoa [36]. The processes of cooling, freezing, and thawing greatly affect the stability and life functions of the membrane cells. The decline in the quality of the spermatozoa above occurs due to damage to the membrane structure during cooling so that the metabolic process of spermatozoa is disturbed [37].

Thus, it is evident that the nutritional content in MOL can provide energy reserves for the spermatozoa which are used for movement after freezing. Vitamins in MOL have functioned as an antioxidant to protect sperms from cool shock due to each treatment in the semen processing.

3.6. Concentration of Bali bulls sperms

Bali bulls sperms concentration before and after supplementation of MOL are shown in figure 2.

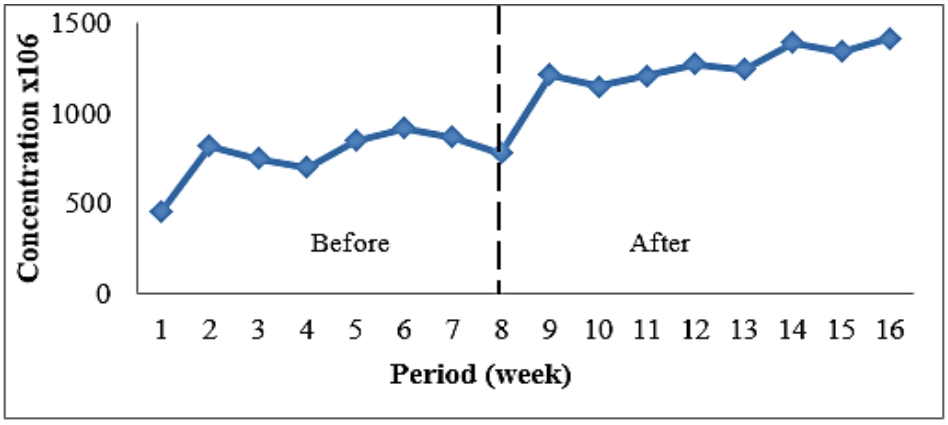

Figure 2. Spermatozoa concentration before and after Moringa supplementation

In this study, the average concentration of bulls' sperms after supplemented with MOL was 1,278 million/mL, significantly ($P < 0.01$) higher than the control group (809,440 million/mL). It appears that the concentration of spermatozoa in each period fluctuated. The sperms concentration of the treated group increased regularly until the end of the study period. Campbell et al. [38] stated that in general, the sperms concentration of adult Bali cattle ranges from 800-1200 million/mL while according to Hafez et al. [15] that a male is considered satisfactory if he has a sperms concentration of >500 million/mL.

One of the factors that influence sperms concentration is the nutritional content of the feeding. According to Salisbury and Van Demark [26], it is clear that, in general, the concentration of sperms in semen is in line with the sexual development and maturity, according to the quality of the feed given.

This shows that MOL supplementation in feeding can improve the quality of the feed so that it will have a good impact on sperms concentration.

It is also suspected that the content of protein or amino acids plays a role in the synthesis of the reproductive hormone which affects libido and scrotal circumference [39]. Apart from that, libido is also related to Vitamin A and fatty acids in Moringa. Other nutrients such as Vitamin C, Vitamin E, Zinc, Calcium, and other minerals which are also contained in Moringa that affect individual motility, progressivity, concentration, and post-thawing motility.

4. Conclusions

The supplementation of MOL in the feed can increase semen quality (volume, mass movement, total sperm motility, and semen concentration) of Bali bulls.

References

[1] Dewi A S, Ondho Y S and Kurnianto E 2012 Kualitas semen berdasarkan umur pada sapi jantan jawa *Anim. Agric. J* **1** 126–33
[2] Ratnawati D, Affandhy L, Pratiwi W C and Prihandini P W 2008 *Pengaruh Pemberian Suplemen Tradisional terhadap Kualitas Semen Pejantan Sapi Bali* Seminar Nasional Teknologi Peternakan dan Veteriner. Loka Penelitian Sapi Potong
[3] Yendraliza 2013 Pengaruh nutrisi dalam pengelolaan reproduksi ternak *Studi Literatur Kutubkhanah* **16** 20-26
[4] Nouman W, Siddiqui M T, Basra S M A, Farooq H, Zubair M and Gull T 2013 Biomass production and nutritional quality of *Moringa oleifera* as a field crop *Turk. J. Agric. Forest.* **37** 410 -19
[5] Nouman W, Basra S M A, Siddiqui M T, Yasmeen A, Gull T and Alcayde M A C 2014 Potential of *Moringa oleifera* L as livestock fodder crop: a review *Turk J Agric Forest.* **38** 1-14
[6] Fitri A, Toharmat T, Astuti D A and Tamura H 2015 The potential use of secondary metabolites in *Moringa oleifera* as an antioxidant source *Med. Pet.* **38** 169-175
[7] Moyo B, Masika, Hugo A and Muchenje V 2011 Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves *African J. Biotech.* **10** 12925-33
[8] Ferreira, Pinheiro P M, Farias D F, Oliveira J T D A and Carvalho A D F U 2008 *Moringa oleifera*: bioactive compounds and nutritional potential *Rev. Nutr.* **21** 431-7
[9] Rajanandh M G and Kavitha J 2010 Quantitative estimation of β-sitosterol, total phenolic and flavonoid compound in the leaves of *Moringa oleifera Int. J. Pharmtech Res.* **2** 1409-14
[10] Madukwe E U, Ezeugwu I O and Eme P E 2013 Nutrient composition and sensory evaluation of dry *Moringa oleifera* aqueous extract *J. Sci. Basic Appl. Res.* **13** 100-102
[11] Abu A H, Ahemen T and Ikpechukwu P 2013 Testicular morphometry and sperm quality of rabbit bucks fed graded levels of *Moringa oleifera* leaf meal (MOLM) *African Journal Online* **13** 49-56
[12] Raji A Y and Njidda A A 2014 Gonadal and extra-gonodal sperm reserves of the red sokoto goats fed *Moringa oleifera* supplemented diets *Int. J. Agri Biosci.* **3** 61-64
[13] Hasrin, Toleng A L, Yusuf M, Sahiruddin and Hasbi H 2020 Conception rate of Bali cows inseminated with semen of the bull supplemented with *Moringa oleifera* Leaf (MOL) powder block *IOP Conf. Ser.: Earth Environ. Sci.* **492** 012071
[14] Rika N, Toleng A L, Yusuf M, Sahiruddin and Ako A 2020 Increasing milk production and milk chemical composition in dairy cows by a supplementation of *Moringa oleifera* leaf (MOL) powder block *IOP Conf. Ser.: Earth Environ. Sci.* **492** 012072
[15] Hafez B, Ax R L, Dally M, Didion B A, Lenz R W, Love C C, Varner da D D and Berlin M E 2000. *Evaluation. In E S E Hafes and Hafez B (Ed.) Reproduction in Farm Animals* Seventh Edition (Philadelphia, USA: Lea and Febiger)
[16] Riwidikdo H 2009 *Statistik Kesehatan* (Jogjakarta: Mitra Cendikia Press)

[17] Partodihardjo S 1989 *Ilmu Reproduksi Hewan* (Penerbit Mutiara: Jakarta)
[18] Toelihere M R 1993 *Inseminasi Buatan Pada Ternak* (Angkasa: Bandung)
[19] Bardan B, Feradis F and Adelina T 2009 Penggunaan air tebu yang dikombinasi dengan kuning telur sebagai pengencer semen sapi Bali *J. Peternakan* **6** 36-43
[20] Partodihardjo S 1992 *Imu Reproduksi Hewan* (Jakarta: Mutiara Sumber Widya)
[21] Ramsiyati D T, Sriyana and Sudarmadi B 2004 Evaluasi Kualitas Semen Sapi Potong pada Berbagai Umur di Peternakan Rakyat *Prosiding Temu Teknis Nasional Tenaga Fungsional Pertanian 2004* (Bogor: Pusat Penelitian dan Pengembangan Peternakan) pp 82 – 87
[22] Dethan A A, Kustono and Hari H 2010 Kualitas dan kuantitas sperma kambing Bligon jantan yang diberi pakan rumput gajah dengan suplementasi tepung darah *Buletin Peternakan* **34** 145-153
[23] Kumar N, Verma R P, Singh L P, Varshney V P and Dass R S 2006 Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) *Reprod. Nutr. Dev.* **46** 663-75
[24] Wahyuningsih A, Dadang M S and Sugiyatno 2013 Pengaruh umur pejantan dan frekuensi penampungan terhadap volume dan motilitas semen segar sapi simmental di Balai Inseminasi Buatan Lembang *J. Ilmiah Peternakan* **1** 947-53
[25] Garner D L and Hafez E S E 2000 Spermatozoa and Seminal Plasma *In Reproduction in Farm Animals* ed E S E Hafes and Hafez (Philadelphia, USA: Lea and Febiger)
[26] Salisbury G W and VanDemark N L 1985 *Fisiologi Reproduksi dan Inseminasi Buatan pada Sapi* Penterjemah R Djanuar (Yogyakarta: Gadjah Mada University Press)
[27] Dewi A S 2004 *Pengaruh Bangsa Terhadap Lingkar Skrotum, Volume Testis, Volume Semen dan Konsentrasi Sperma pada Sapi Potong* (Semarang: Fakultas Peternakan Universitas Diponegoro)
[28] Sarastina, Susilawati T and Ciptadi G 2006 Analisa beberapa parameter motilitas spermatozoa pada berbagai bangsa sapi menggunakan computer assisted semen analysis (CASA) *J. Ternak Tropika* **6** 1-12
[29] Cheah Y and Yang W 2011 Functions of essential nutrition for high quality spermatogenesis *Advan. Biosci. Biotechnol.* **2** 182-97
[30] Kathryn A 2011 *The Nutrient Content of Moringa oleifera Leaves* (PA: Messiah College Department of Nutrition and Dietetics)
[31] Zhou X, Liu F, and Zhai S 2007 Effect of L-car-nitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: A systematic review *Asia-Pacific J. Clinic. Nutr.* **16** 383-90
[32] Chinoy N J, Seqeurina E and Narayana M V 1991 Effects of vitamin C and calciom on the reversibility of fluoridde-indecute alterations in spermatozoa of the rabbits *Floride* **24** 29-39
[33] Aurich J E, Schoneher U, Hoppe H and Aurich C 1997 Effect of antioxidants on motility and membrane integrity of chilled-stored stallion semen *Theriogenology* **48** 185-192
[34] Aslam H A, Dasrul, dan Rosmaidar 2014 Pengaruh penambahan vitamin C dalam pengencer andromed® terhadap persentase motilitas dan membran plasma utuh spermatozoa sapi Aceh setelah pembekuan *J. Med Veterinaria* **8** 20-26
[35] Hafes E S E 2004 X- and Y-Chromosome-Bearing *Spermatozoa* In *Reproduction in Farm Animals* E S E Hafes and Hafez B (Ed.) Seventh Edition (Philadelphia, USA: Lea and Febiger)
[36] Suharyati S dan Hartono M 2011 Preservasi dan riopreservasi semen sapi limousin dalam berbagai bahan pengencer *J. Kedokteran Hewan* **5** 72-76
[37] Susilawati T, Suyadi, Nuryadi, Isnaini N dan Wahyuningsih S 1993 *Kualitas Semen Sapi Fries Holland dan Sapi Bali Pada Berbagai Umur dan Berat Badan* Laporan Penelitian Fakultas (Malang: Peternakan Universitas Brawijaya)
[38] Campbell J R, Campbell K L and Kenealy M D 2003 Artificial Insemination In: *Animal Science* 46 (New York: Mc-Graw Hill)
[39] Guyton 1997 *Sistem Hormon dan Fisiologi Tubuh* (Bandung: Yrama Widya)