Journal of Animal & Plant Sciences, 33(3): 2023, Page: 722-726
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.3.0665
Short Communication

EFFECTS OF SUPPLEMENTATION OF GUAVA JUICE (*Psidium guajava L.)* TO THE ANDROMED DILUENT ON THE QUALITY OF INDIGENOUS CHICKEN SEMEN STORED AT ROOM TEMPERATURE

F. K. Nisaa[1], F. Andri[2], M. N. Ihsan[2], and N. Isnaini[2*]

[1]Undergraduate Program of Animal Science, [2]Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia
*Corresponding author's email: nurulisna@ub.ac.id

ABSTRACT

Indigenous chicken serve as a highly valuable germplasm for the development of the poultry industry in Indonesia. They also play an important role in rural communities as a source of meat, eggs, and additional income. This study evaluated the semen quality of indigenous chicken in the Andromed diluent supplemented with different levels of guava juice during storage at room temperature (24-25ºC) for 0, 30, 60 or 90 minutes. The semen was collected from an indigenous rooster, aged 2.5 years with body weight of 2.5 kg. A total of 10 ejaculates were collected from this rooster; the frequency of semen collection was twice a week. The semen was diluted in the Andromed diluent supplemented with guava juice at the level of 0 (control), 2, 4, or 6%. Evaluation of semen quality parameters included sperm motility, live sperm, and morphologically abnormal sperm. Data were analyzed by using two-way analysis of variance followed by Duncan Multiple Range Test to compare means. Results showed that regardless of storage time, the use of 6% guava juice had significantly higher (P<0.05) sperm motility and live sperm, but lower abnormal sperm as compared to other treatments. It was also found that regardless of guava juice level, the longer storage time continually decreased (P<0.05) the sperm motility and live sperm, but increased (P<0.05) abnormal sperm. Results also showed that there were interaction effects (P<0.05) between the level of guava juice and storage time on the sperm motility and live sperm. This study provides evidence that the addition of 6% guava juice to the Andromed diluent results in better semen quality for artificial insemination up to 60 minutes of storage at room temperature.

Keywords: Andromed, guava juice, room temperature, semen quality, indigenous chicken.

Published first online February 10, 2023 Published final June 18, 2023

INTRODUCTION

Indigenous chicken serve as a highly valuable germplasm for the development of the poultry industry in Indonesia. They also play an important role in rural communities as a source of meat, eggs, and additional income (Sumantri *et al*., 2020). Indigenous chicken have long been known by the community and have been widely spread throughout Indonesia, especially in the rural areas. Some of the advantages of indigenous chicken rearing include ease of maintenance, high adaptability to the environment and feed, high survival rate, and preferred taste of both of meat and eggs compared to commercial chicken (Nafiu *et al*., 2020).

The chicken semen has high sperm concentration with a very small ejaculate volume. For more efficient use of chicken semen in terms of insemination of more female, a good diluent is needed (Łukaszewicz *et al*., 2020). The existing problem during the process of semen collection, dilution, and storage is the production of free radicals as a result of metabolic activities of spermatozoa. The production of free radicals may have negative effects on the sperm quality (Mehaisen *et al*., 2020; Salehi *et al*., 2020; Najafi *et al*., 2021).

During the metabolic process, spermatozoa are not able to rely solely on the endogenous antioxidants, but also require exogenous antioxidants to be added, namely in the form of vitamin C, vitamin E, polyphenols, and carotenoids (Gopi *et al*., 2020; Leão *et al*., 2021). Guava (*Psidium guajava* L.) is a fruit that can also be used as a functional food because it possesses antioxidant, antimicrobial, antiviral and anticancer activities (Kapoor *et al*., 2020). The functional properties of guava are due to its high contents of vitamin C so that it can prevent the adverse effects of free radicals such as superoxide anion radicals and hydroxyl free radicals (Siwarungson *et al*., 2013). Other advantages of guava are due to the low price, relatively easy to obtain, and not dependent on the season.

A previous study has shown that the use of guava extract can maintain intact plasma membrane and intact acrosome hood of Bali bull spermatozoa during storage for six days (Marawali *et al*., 2020).However, there are relatively few reports regardeing the use of guava products as an antioxidant source for chicken spermatozoa.Therefore, this study was conducted to evaluate the effects of adding various levels of guava juice in the Andromed diluent on the semen quality of indigenous chicken during storage upto 90 minutes at room temperature.

MATERIALS AND METHODS

Location and time: This research was carried out at the Laboratory of Animal Reproduction and Health, College of Agricultural Extension (STPP) Malang, Indonesia during January and February, 2018.

Experimental design: The material used was fresh semen collected from an indigenous rooster, aged 2.5 years with body weight of 2.5 kg. This rooster was selected on the basis of good genetic performance, no history of the disease, high libido, and production of good quality semen. This rooster was maintained intensively in a cage and had trained for semen collection. The frequency of semen collection was twice a week by using female teaser method (Iswati *et al*., 2018). A total of 10 ejaculates were collected from this rooster over a period of five weeks.

Soon after collection, fresh semen volume was measured using a scaled tube (Isnaini *et al*., 2020) along with the observation on the color and consistency. pH of semen was measured using a pH indicator paper with the scale ranged from 1 to 14. Sperm concentration was measured using a spectrophotometer (Bayu *et al*., 2020). The sperm motility was assessed under a light microscope with 400 times magnification (Isnaini *et al*., 2019). Live sperm and morphologically abnormal sperm percentages were estimated using eosin-nigrosin staining procedure, as described previously (Wahjuningsih *et al*., 2019).

The method used was an experiment in a randomized complete block design with 4 treatments and 10 replications. The time of semen collection was considered as blocks. The diluent used was andromed diluent supplemented with different levels of guava juice according to the following treatments: T0: 100% Andromed, T1: 98% Andromed + 2% guava juice (v/v), T2: 96% Andromed + 4% guava juice (v/v), and T3: 94% Andromed + 6% guava juice (v/v). One ml of diluent was prepared according to the treatments. After that 0.1 ml of semen were added to the respective diluent and homogenized slowly. The semen was then stored under room temperature (24-25^{o}C) for up to 90 minutes. Evaluation of semen quality during storage included individual sperm motility (Isnaini *et al*., 2019), live sperm, and morphologically abnormal sperm, as described above. These evaluations were done at 0, 30, 60 and 90 minutes of storage.

Statistical analysis: Data were analyzed through two-way analysis of variance technique. The first factor was fourlevels of guava juice (0, 2, 4, and 6%), while the second factor was four storage periods (0, 30, 60 and 90 minutes). Data with significant effect (P<0.05) were further analyzed using Duncan Multiple Range Test (Duncan, 1955). All statistical analysis was conducted by using SPSS version 22 (IBM SPSS, NY, USA).

RESULTS

Fresh semen quality: Table 1 shows the fresh semen quality of indigenous rooster used in this study. The color of fresh semen of indigenous rooster in this study was milky white, while the consistency of semen was thick. Generally, fresh semen of indigenous rooster used in this study had good quality.

Table 1. Fresh semen quality of indigenous rooster.

Parameter	Average	SD	Max	Min
Macroscopic				
Semen color	Milky white	NA	NA	NA
Semen consistency	Thick	NA	NA	NA
Semen volume (ml)	0.53	0.08	0.60	0.40
Semen pH	7.80	0.63	9.00	7.00
Microscopic				
Sperm concentration (10^9 spermatozoa/ml)	3.97	0.19	4.39	3.75
Sperm motility (%)	86.00	2.10	90.00	85.00
Live sperm (%)	89.08	3.09	95.40	86.20
Abnormal sperm (%)	3.77	0.98	5.47	2.60

SD: standard deviation, Max: maximum value, Min: minimum value, NA: not analyzed

Semen quality during storage

Sperm motility: Table 2 shows that regardless of storage time, guava juice addition significantly improved (P<0.05) sperm motility, with the use of 6% guava juice had significantly higher sperm motility as compared to other treatments. It was also found that sperm motility decreased (P<0.05) as the storage duration was increased, regardless of guava juice treatment. The addition of guava juice in the Andromed diluent also showed interaction effect with storage time on the sperm motility of indigenous rooster. During 0 minutes of storage, sperm motility did not differ among treatments. However, after 30, 60, and 90 minutes of storage, guava juice addition significantly (P<0.05) improved sperm motility, with the highest sperm motility was recorded with the use of 6% guava juice.

Table 2. The sperm motility, live sperm, and abnormal sperm of indigenous rooster with the addition of guava juice in the Andromed diluent during storage at room temperature.

	Sperm motility (%)	Live sperm (%)	Abnormal sperm (%)
Guava juice (%) (G)			
0	48.64±23.36^{a}	53.34±22.51^{a}	8.37±2.69^{d}
2	53.99±21.73^{b}	58.84±20.11^{b}	7.11±2.28^{c}
4	56.70±19.66^{c}	60.94±19.29^{b}	6.28±2.03^{b}
6	59.90±18.36^{d}	65.07±17.46^{c}	5.58±1.73^{a}
Storage time (min) (S)			
0	80.68±2.09^{d}	83.40±2.03^{d}	4.81±1.09^{a}
30	64.68±5.81^{c}	68.93±5.73^{c}	5.89±1.22^{b}
60	47.34±6.84^{b}	53.68±8.66^{b}	7.12±2.02^{c}
90	26.54±7.95^{a}	32.20±8.54^{a}	9.54±2.12^{d}
G x S			
0 x 0	78.96±2.28^{k}	82.31±2.22^{j}	5.79±0.76
2 x 0	80.86±1.67^{k}	82.69±1.94^{j}	4.98±0.65
4 x 0	80.96±1.63^{k}	83.65±1.90^{j}	4.55±1.13
6 x 0	81.93±1.76^{k}	84.93±1.05^{j}	3.91±0.87
0 x 30	57.23±6.07^{h}	62.79±2.67^{g}	7.06±1.17
2 x 30	64.36±1.55^{i}	68.12±3.00^{h}	6.07±0.94
4 x 30	66.93±1.29^{ij}	69.89±5.58^{h}	5.51±0.92
6 x 30	70.20±1.99^{j}	74.92±3.39^{i}	4.91±0.73
0 x 60	41.74±3.50^{e}	45.68±5.37^{d}	8.70±2.00
2 x 60	46.70±6.25^{f}	53.24±8.04^{e}	7.38±1.96
4 x 60	48.48±6.62^{f}	56.30±7.32^{ef}	6.62±1.90
6 x 60	52.45±6.48^{g}	59.49±7.86^{fg}	5.76±1.06
0 x 90	16.62±3.73^{a}	22.59±2.82^{a}	11.93±1.37
2 x 90	24.05±4.93^{b}	31.32±8.38^{b}	10.00±1.34
4 x 90	30.45±3.93^{c}	33.94±5.29^{b}	8.46±1.64
6 x 90	35.05±2.51^{d}	40.93±4.32^{c}	7.75±1.28
P value			
G	0.000	0.000	0.000
S	0.000	0.000	0.000
G x S	0.000	0.002	0.201

$^{a-k}$ different superscripts within a column showed a significant difference at P<0.05

Live sperm: Table 2 also shows that guava juice inclusion significantly improved (P<0.05) live sperm percentage of indigenous roosters, regardless of storage time. The inclusion of 6% guava juice had significantly higher (P<0.05) live sperm as compared to other treatments. Regardless of guava juice addition, live sperm of indigenous rooster continually decreased (P<0.05) along with the longer storage time. Results also showed that guava juice inclusion interacted with storage time on the live sperm percentage of indigenous roosters. At initial storage time (0 minutes), percentage of live sperm was similar among treatments. However, it significantly differed (P<0.05) among treatments when stored for 30, 60, and 90 minutes. The supplementation of 6% guava juice had significantly higher (P<0.05) live sperm as compared to other treatments, particularly at 30 and 90 minutes of storage.

Abnormal sperm: In this study, both of guava juice supplementation and storage time had significant effects on morphologically abnormal sperm percentage (Table 2). However, there was no interaction between these two factors for sperm abnormality. Regardless of storage time, the supplementation of 6% guava juice to the Andromed diluent had significantly lower (P<0.05) abnormal sperm as compared to other treatments. Similarly, regardless of guava juice addition, percentage of abnormal sperm significantly increased (P<0.05) as the storage time was increased.

DISCUSSION

The average volume of fresh ejaculate (0.53 ml) found in this study isalmost comparable to the average semen volume of 0.56 ml for indigenous roosters reported by Iswati *et al*. (2018). The pH of fresh semen in this study was also almost similar to that reported by Iswati *et al*. (2018), who reported that the average pH of the indigenous rooster semen was 7.50. The sperm motility percentage of fresh semen of indigenous rooster was between 85 and 90%, which is generally considered good (Rad *et al*., 2016).

In this study, the sperm motility and live sperm percentages decreased, but abnormal sperm percentage increased significantly (P<0.05) after 30 minutes of storage and this deterioration continued with the increase in storage time. This may be related to the presence of lipid peroxidation as a result of the free radical attack during storage at room temperature. Hammerstedt (1993) stated that free radicals can cause lipid peroxidation, thus killing sperm and reduce live sperm percentage. The free radicals also have adverse effects on the unsaturated fatty acids, which are the main components of spermatozoa plasma membrane phospholipids. Damaged plasma membrane leads to the increase in abnormal sperm. Besides, damaged plasma membrane also leads to metabolic disturbances so that the ATP production as a source of energy is reduced. Since the sperm motility highly depends on the energy supply, the ATP reduction will ultimately decrease sperm motility.

The addition of guava juice, particularly at the level of 6%, consistently showed significantly higher sperm motility and live sperm, but lower morphologically anormal sperm as compared to control. These results may be due to the presence of the antioxidant compounds in guava juice in the form of vitamin C and flavonoids which act as neutralizers of free radicals formed due to the metabolic activity of spermatozoa. The neutralization of free radicals, in turn, could maintain the sperm motility. According to Leão *et al*. (2021), vitamin C, vitamin E, and lycopene arethe antioxidants which could bind the oxygen radicals contained in the cells, and may prevent the process of lipid peroxidation in the mitochondrial membrane, resulting in the inhibition of glycolysis and maintenance of sperm motility.

Results of the present study indicated that the semen quality of indigenous rooster which was dilutedwith Andromed diluent supplemented with various levels of guava juice showed good quality up to 60 minutes of storage because it wasstill suitable for use in artificial insemination. Masoudi *et al*. (2019) suggested that the sperm motility value which isfeasible to be used for artificial insemination should be above 40%.

Based on the results of this study, it can be concluded that the inclusion of guava juice (*Psidium guajava* L.) into Andromed diluent could maintain the semen quality of indigenous rooster up to 60 minutes of storage at room temperature. The addition of guava juice at the level of 6% in Andromed diluent resulted in the best semen quality although other treatments also maintained desirable semen quality for use in artificial insemination for up to 60 minutes.

REFERENCES

Bayu, S., N. Isnaini, and S.I. Adie (2020). Influence of bull age on fresh semen traits of Bali cattle. Russ. J. Agric. Soc.-Econ. Sci. 98(2): 27-30.

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics. 11(1): 1-42.

Gopi, M., P.V. Beulah, G. Prabakar, and J. Mohan (2020). Polyphenols as an antioxidant agent improves chicken sperm qualities during cold storage. J. Anim. Plant Sci. 30(6): 1653-1658.

Hammerstedt, R.H. (1993). Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: A review of the effect on storage preservation system. J. Reprod. Fertil. Dev. 5: 675-690.

Isnaini, N., M.N. Ihsan, and S. Wahjuningsih (2019). Mangosteen peel extract in Tris-egg yolk extender improves fertility of cryopreserved goat sperm. Livest. Res. Rural Dev. 31(4): 53.

Isnaini, N., G. Ciptadi, E. Herwijanti, N.I.P. Walidah, and M.W.S.N. Putra (2020). Effects of seasons and environmental conditions on semen quality of Senduro goats reared under tropical climate. Turkish J. Vet. Anim. Sci. 44(3): 594-599.

Iswati, N. Isnaini, and T. Susilawati (2018). Effect of addition of glutathione in diluent Ringer's on spermatozoa quality of domestic chicken during cold storage. Asian J. Microbiol. Biotechnol. Environ. Sci. 20(1): 12-20.

Kapoor, S., N. Gandhi, and A. Kapoor (2020). Guava (*Psidium guajava*). In: Nayik, G.A. and A. Gull (eds.). Antioxidants in Fruits: Properties and Health Benefits. Springer; Singapore. 227-249.

Leão, A.P.A., A.V. de Souza, N.F. Mesquita, L.J. Pereira, and M.G. Zangeronimo (2021). Antioxidant enrichment of rooster semen extenders–A systematic review. Res. Vet. Sci. 136: 111-118.

Łukaszewicz, E., A. Jerysz, and A. Kowalczyk (2020). Effect of semen extenders on viability of ISA Brown and Hubbard Flex roosters' sperm stored for 24 h. Poult. Sci. 99(5): 2766-2774.

Marawali, A., M.S. Abdullah, W.M. Nalley, T.M. Hine, and Y.L. Henuk (2020). Preservation of bovine semen using egg yolk substituted with extract guava in water from yellow coconut on the semen quality and fertility of Bali cattle. In: IOP Conf. Ser. Earth Environ. Sci. 454: p. 012071.

Masoudi, R., M. Sharafi, and L. Pourazadi (2019). Improvement of rooster semen quality using coenzyme Q10 during cooling storage in the Lake extender. Cryobiology. 88: 87-91.

Mehaisen, G.M., A. Partyka, Z. Ligocka, and W. Niżański (2020). Cryoprotective effect of melatonin supplementation on post-thawed rooster sperm quality. Anim. Reprod. Sci. 212:106238.

Nafiu, L.O., M. Abadi, and I. Wati (2020). Characteristics of qualitative and quantitative properties of chicken village in the Sub-District Lasusua, North Kolaka District. ANJORO: Int. J. Agric. Bus. 1(2): 67-74.

Najafi, A., H.D. Kia, and H. Hamishehkar (2021). Does alpha-lipoic acid–loaded nanostructured lipid carriers improve post-thawed sperm quality and ameliorate apoptosis-related genes of rooster sperm? Poult. Sci. 100(1): 357-365.

Rad, H.M., M. Eslami, and A. Ghanie (2016). Palmitoleate enhances quality of rooster semen during chilled storage. Anim. Reprod. Sci. 165: 38-45.

Salehi, M., A.H. Mahdavi, M. Sharafi, and A. Shahverdi (2020). Cryopreservation of rooster semen: Evidence for the epigenetic modifications of thawed sperm. Theriogenology. 142: 15-25.

Siwarungson, N., I. Ali, and T. Damsud (2013). Comparative analysis of antioxidant and antimelanogenesis properties of three local guava (*Psidium guajava* L.) varieties of Thailand, via different extraction solvents. J. Food Meas. Charact. 7(4): 207-214.

Sumantri, C., I. Khaerunnisa, and A. Gunawan (2020). The genetic quality improvement of native and local chickens to increase production and meat quality in order to build the Indonesian chicken industry. In: IOP Conf. Ser. Earth Environ. Sci. 492: p.012099.

Wahjuningsih, S., G. Ciptadi, M.N. Ihsan, N. Isnaini, and S. Rahayu (2019). Supplementation of *Moringa oleifera* leaves' extract in Tris-egg yolk extender on the quality and fertility of cryopreserved Senduro goat sperm. Livest. Res. Rural Dev. 31(12):185.