

The protective effect of pumpkin seed extract (*Cucurbita moschata*) on the characteristics of local rooster sperm exposed to heat

Siti Eliana Rochmi ^{1, 2}, Sri Agus Sudjarwo ^{3, *}, Rochmah Kurnijasanti ³, Rimayanti ⁴, Sri Pantja Madyawati ⁴, Hani Plumeriastuti ⁵, Mohammad Anam Al Arif ⁶, M Gandul Atik Yuliani ³, Miyayu Soneta Sofyan ², Herinda Pertiwi ², Agung Budianto Achmad ² and Supriyadi ⁷

¹ Doctoral Student of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

² Veterinary Technology Program, Department of Health, Faculty of Vocational Studies Universitas Airlangga, Surabaya, Indonesia.

³ Veterinary Basic Medical Sciences Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁴ Veterinary Reproduction Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁵ Veterinary Pathology Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁶ Division of Animal Husbandry Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁷ Student in Master's Degree of Reproductive Biology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

GSC Biological and Pharmaceutical Sciences, 2025, 30(03), 094-098

Publication history: Received on 15 January 2025; revised on 27 February 2025; accepted on 02 March 2025

Article DOI: <https://doi.org/10.30574/gscbps.2025.30.3.0080>

Abstract

Pumpkin seeds have been associated with myriad of medicinal uses in different part of the world. The pumpkin seed is a rich natural source of antioxidant. Till date researchers reported significant potential of herbal drugs employed in various traditional, complementary and alternative systems. The pharmacological activity of several medicinal plants has been scientifically documented. The present review is useful for up-to date investigations on the medicinal activity of *Cucurbita moschata* furthermore, we assessed the possible protective efficacy of pumpkin seed extract on sperm characteristics. The study aimed to improved quality of spermatozoa by giving extracted pumpkin seeds in domestic rooster. Different level of extract pumpkin seeds (T0: 0 mg/Kg BB, T1:1600 mg/Kg BB, T2: 3200 mg/Kg BB, and T3: 6400 mg/Kg BB). Volume, pH, Concentration, motility, viability, abnormality of spermatozoa were measured twice a week after exposed to heat. This research indicate that supplementation of extract Pumpkin seed could significantly improve quality of rooster spermatozoa, therefore it could support productivity of domestic rooster in tropical area.

Keywords: Chicken; Heat Stress; Healty; Pumpkin Seed; Spermatozoa

1. Introduction

Indonesia has at least 34 type of local chicken in Indonesia. Thus, the most popular and reared almost thoroughot the entire country is kampung chicken (FAO, 2008; Henuk et al, 2015). However, it has slow sexual maturity, low productivity, and relatively expensive price because the high demand which not offset by increased production (Hambu et al, 2016). Increasing the quality and quantity of sperm is one of the problem solving in the context of developing national farms and conserve the plasmagerm.

The Spermatozoa and Seminal plasma (semen) is important in poultry breeding. Poultry semen contains $3-8 \times 10^9$ /ml spermatozoa for roosters. Good semen quality is a crucial tool for the rapid improvement of fertility by allowing

* Corresponding author: Sri Agus Sudjarwo

maximum use of genetically improved roosters and evaluation of semen quality is crucial before the selection of breeding rooster used for artificial insemination (AI) (Ayeneshet *et al.*, 2024).

Artificial insemination (AI) in the poultry is the method of choice to increase poultry production, genetic improvement and control of venereal diseases (Dhama *et al.*, 2014; Getachew, 2016; Kharayat *et al.*, 2016). The Successfull of the method depends on strain of chicken, age of chicken, semen quality, diluent for sperm, dose of sperm, and storage of semen (Hambu *et al.*, 2016; Mohan *et al.*, 2018; Rochmi and Sofyan, 2019). Both of Fresh and cryopreservation semen can be used for AI in the poultry (Th'elie *et al.*, 2019). Cryopreservation semen can protect the plasmagerm in danger of loss. However, chicken sperm is more sensitive to the freezing process and their quality post thawing is also relatively low compared with the mammalian species (Mosca *et al.*, 2016; Malik *et al.*, 2019).

The environmental temperature is an important factor of semen quality and fertility of roosters intropical countries where the avian are mostly raised in opensided poultry house. It has long been reported that semen quality is decreased during summer or on exposure to high ambient temperature. The heat exposure might have resulted innuclear abnormality leading to declined fertility (Shanmugam *et al.*, 2015). The addition of dietary supplements the pumpkin seed extract which serve as antioxidant, immunomodulator, and mineral Zn which containing of pumpkin seed extract can protect testicular damage due to exposure to heat, heavy metals, and fluoride (Rowe *et al.*, 2013).

Pumpkin (*Cucurbita sp.*) is a fruit vegetable, native to the Western Hemisphere and easily cultivated in Tropical Asia countries such as Indonesia, Malaysia, and Philippines (Lestari and Meiyanto, 2018). There are many varieties of pumpkin such as *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita ficifolia*, and *Cucurbita turbaniformis* in which *Cucurbita moschata* exhibits the widest variation in Indonesia (Gemrot *et al.*, 2006). Although the pumpkin itself has various benefits, the pumpkin seeds have been the focus of interest in the last few years in the field of diet and disease research due to the emerging various active components. In many studies reported that pumpkin seed are a rich natural source of phytosterols (Phillips *et al.*, 2005), proteins, polyunsaturated fatty acids (Sabudak, 2007), antioxidant vitamins, carotenoids and tocopherols (Stevenson *et al.*, 2007) and various elements (Glew *et al.*, 2006) due to these components are attributed providing many health benefits. Pumpkin seed extract (*Cucurbita moschata*) has shown antioxidant effects through the lipoxygenase inhibitory pathway and its antioxidant ability can be examined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) analysis and also inhibits lipid peroxidation (Dowidar *et al.*, 2020).

2. Material and methods

A total of 27 male domestic rooster aged one year old were used as sampel divided into four treatment groups (T0, T1, T2, and T3) and negative control. Different level of pumpkin seeds extract (T0: 0 mg/Kg BB, T1:1600 mg/Kg BB, T2: 3200 mg/Kg BB, and T3: 6400 mg/Kg BB). Semens were collected once a week by abdominal massage method, then evaluated for volume, color, pH, consistency, concentration, motility, viability, abnormality. good quality cement (70% progressive motility) is then made into one in a sterile test tube. The data were analyzed by Analysis of Variance (ANAVA) utilized SPSS version 25.00 software.

2.1. Study Period and Location

Semen quality test was performed in the Laboratory of Veterinary Technology, Faculty of Vocational Studies, Universitas Airlangga, Surabaya. Pumpkin Seeds (*Cucurbita moschata*) were determined at the Tuban Regency, East Java, Indonesia. All procedure for extraction was performed in the Laboratory of Faculty of Pharmacy, Universitas Airlangga, Surabaya. This study performed from Agustus 2023 to January 2024.

3. Results and Discussion

The extract pumpkin seed contain mineral Zn which can protect testicular damage due to exposure to heat, heavy metals, and fluoride. The results of spermatozoa from domestic rooster exposed to heat stress and treated with pumpkin seeds extract showed positive results both macroscopically and microscopically show in Table 1. It is caused by mineral Zn from Extract pumpkin seed allowing for the optimal spermatogenesis, good conditions are created from the seminiferous tubules to the epididymis that can maintain spermatozoa life optimally (Mustafa *et al.*, 2017). This is related to the function of hormones to maintain complementary genital organs which will produce seminal plasma for metabolism of spermatozoa and as a source of food substances for spermatozoa to live.

Table 1 Means of quality of rooster sperm were treated extract pumpkin seed

Characteristics	Means $\pm$ SD				
Macroscopic	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Control -
Volume (ml)	0,298 $\pm$ 0,054 ^a	0,408 $\pm$ 0,620 ^b	0,692 $\pm$ 0,062 ^c	0,745 $\pm$ 0,049 ^{cd}	0,798 $\pm$ 0,036 ^d
Color	White	Cream	Cream	Cream	Cream
Consistency	Thin	Thick	Thick	Thick	Thick
Odor	Typical	Typical	Typical	Typical	Typical
pH	4,722 $\pm$ 0,461	5,611 $\pm$ 0,502	6,778 $\pm$ 0,428	7 $\pm$ 0	7 $\pm$ 0
Microscopic					
Mass motility	+(1) $\pm$ 0,00	++(2) $\pm$ 0,00	+++ (3) $\pm$ 0,00	+++ (3) $\pm$ 0,00	+++ (3) $\pm$ 0,00
Sperm mobility (%)	20,111 $\pm$ 2,541 ^a	51,889 $\pm$ 3,251 ^b	81,611 $\pm$ 3,109 ^c	90,389 $\pm$ 1,577 ^d	92,111 $\pm$ 2,934 ^d
Concentration (10 ⁹ cells/ml)	2,498 $\pm$ 0,283 ^a	3,073 $\pm$ 0,245 ^b	3,147 $\pm$ 0,160 ^{bc}	3,236 $\pm$ 0,190 ^{bc}	3,301 $\pm$ 0,174 ^c
Sperm viability (%)	10,500 $\pm$ 1,863 ^a	43,278 $\pm$ 2,164 ^b	73,472 $\pm$ 2,440 ^c	90,000 $\pm$ 2,29 ^d	91,944 $\pm$ 3,459 ^d
Sperm abnormality (%)	13,722 $\pm$ 2,045 ^d	11,083 $\pm$ 1,717 ^c	7,417 $\pm$ 1,468 ^b	5,917 $\pm$ 1,407 ^b	2,778 $\pm$ 1,228 ^a

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$).

Spermatozoa produced after heat stress exposure is lower than normal. Heat stress in domestic rooster can cause process barriers in the hypothalamus and cause hormonal disorders. Hormonal disorders can cause ldyg cell failure in secreting the hormone testosterone, thus inhibiting spermatogenesis. Acute heat stress can increase HSP70, HSPD1, and HSPA5 in the testicles. The expression of these genes is involved in the process of apoptosis (Murugesan *et al.*, 2017).

The motility of spermatozoa exposed by heat stress decreased compared with before condition (Tabel I). This is because heat stress in domestic rooster can increase HSP70 in the brain (Zhang *et al.*, 2014). Heat stress in poultry causes endocrine dysfunction, reduced antioxidant capacity, and disrupted calcium, phosphorus and mineral zinc balance (Ma *et al.*, 2014). The testosterone hormone correlates negatively with corticosterone. Therefore mineral Zn can improve secretion of this hormones. Testosterone in epididymis is converted to dihydrotestosterone (DHT) by the enzyme 5 α reductase. The function of DHT to eliminate cytoplasmic droplet in the spermatozoa, therefore spermatozoa can released from the epididymis and have high motility. If testosterone and DHT in the epididymis increase, the process of removal of the cytoplasmic droplet will improve and spermatozoa that emerge from the epididymis will also increase of motility. Mineral Zn can influence of motility, and viability bufallo spermatozoa (Alavl-Shoushtari *et al.*, 2009).

In other side, The highest dosages of this pumpkin seed extract (T3) showed decrease of spermatozoa concentration, motility, viability, and abnormalities significantly. It occurred because of Zn imbalance in seminal plasma or spermatozoa may have a negative impact on seminal abnormalities and rised of oxidative stress (Heidari *et al.*, 2018).

4. Conclusion

Supplementation of extrct pumpkin seed extract (dose 3200 mg/Kg BB) can increase quality sperm. although it requires further research related to the mechanism.

Compliance with ethical standards

Acknowledgments

The authors express their gratitude to the Universitas Airlangga, Surabaya, Indonesia.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

We conducted all animal experiments based on the guidelines approved by the Animal Care and Use Committee (Approval number: 1.KEH.162.10.2023), and all procedures of experiments have been agreed upon by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

References

- [1] Alavi-Shoushtari, S.M., S.A. Rezal., and A. Khaki. Effects Of The Seminal Plasma Zinc Content And Catalase Activity On The Semen Quality Of Water Buffalo (*Bubalus bubalis*) Bulls. *Pak J Biol Sci.* 2009; 12 (2): 134-139. DOI: 10.3923/pjbs.2009.134.139
- [2] Ayeneshet, B., M. Taye, W. Esatu, and A. Tsefa. Comparative analysis of semen quality and fertility in diverse rooster breeds: a systematic review, *World's Poult., Sci. J.* 2024; 1(1): 1-29.
- [3] Dhama, K., R. Tiwari, R.U. Khan, S. Chakraborty, M. Gopi, K. Kathik, M. Saminathan, P.A. Desingu, and L.T. Sunkara. Growth Promoters and Novel Feed Additives Improving Poultry Production and Health, *Bioactive Principles and Beneficial Applications: The Trends and Advances: A Review.* *Int. J. of Pharm.* 2014; 10(3):129-159
- [4] Dowidar, M.F., A.I. Ahmed, and H.R. Mohamed. The Critical Nutraceutical Role of Pumpkin Seeds in Human and Animal Health: An Updated Review, *Zagazig Vet J.* 2020; 48(2):199-212.
- [5] Gemrot, F., Barouh, N., Vieu, J. P., Pioch, D. and Montet, D., 2006, Effect of Roasting on Tocopherols of Gourd Seeds (*Cucurbita pepo*), *Gras. Aceit.* 2006; 57: 409–414
- [6] Getachew, T. A Review Article of Artificial Insemination in Poultry. *World's Vet. J.* 2016; 6(1):25-33
- [7] Glew RH, Glew RS, Chuang LT, Huang YS, Millson M. Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp) and *Cyperus*: esculent till nuts in the Republic of Niger. *Plant Foods Hum Nut.* 2006; 61: 51-56.
- [8] Hambu, E.K., R.I. Arifiantini, B. Purwantara, and S. Darwati. Raw Semen Characteristics of Three Different Indonesian Local Roosters. *Animal Production.* 2016; 18(3):165-172
- [9] Heidari, J., J. Seifdavati, H. Mohebodini, R.S. Sharifi, and H.A. Benemmar.. Effect of Nano Zinc Oxide on Post-Thaw Variables and Oxidative Status of Moghani Ram Semen. *Kafkas Univ Vet Fak Derg.* 2013; 25(1): 71-76.
- [10] Henuk, Y.L., J.F. Bale-Therik, G.A.K. Dewi, and C.A. Bailey. Native chickens and their production systems in Indonesia. *Khon Ken Agr. J.*, 2015; 43 Suppl.2
- [11] Kharayat, N.S., G.R. Chaudhary, R. Katiyar, B. Balmurugan, M. Patel, S. Uniyal, M. Raza, and G.K. Mishra. Significance of Artificial Insemination in Poultry. *Research & Reviews: J. of Vet. Sci. and Tech.* 2016; 5(1):1-5
- [12] Lestari, B., and E. Meiyanto. A Review: The Emerging Nutraceutical Potential of Pumpkin Seeds. *Indones. J. Cancer Chemoprevent.* 2018; 9(2): 92-101
- [13] Ma X, Y. Lin, H. Zhang, W. Chen, S. Wang, D. Ruan, and Z. Jiang. Heat stress impairs the nutritional metabolism and reduces the productivity of egg-laying ducks. *Anim Reprod Sci.* 2014; 145:182-190.
- [14] Malik, A., A. Indah, M. I. Zakir, Sakiman, and S. Nugroho. Cryopreservative Effect of Adding a Honey Solution to Native Chicken Spermatozoa. *Adv. In Anim. And Vet. Sci.* 2019; 7(4):261-264
- [15] Mosca, F., L. Zaniboni, A.A. Sayed, M. Madeddu, N. Iaffaldano, and S. Cerolini. Effect of dimethylacetamide and N-methylacetamide on the quality and fertility of frozen/thawed chicken semen. *Poult. Sci.* 2019; 98:6071-6077
- [16] Mohan, J., S.K. Sharma, G. Kolluri, and K. Dhama. History of artificial insemination in poultry, its components and significance. *World's Pout. Sci. J.* 2018; 74(3):475-488
- [17] Mustafa, Dasrul, Yaman, M.A., Wahyuni, S., and Sabri, M. Effect addition of fermented feed with the multi-enzyme digestion and vitamin E in commercial feed on improvement the quality of semen arab chicken. *Agripet.* 2017; 17(1):43-52.
- [18] Murugesan S., Ullengala, R., and Amirthalingam, V. Heat Shock Protein and Thermal Stress in Chicken in *Book: Heat Shock Proteins in Veterinary Medicine and Sciences.* Springer : USA. pp. 2017; 179-193
- [19] Phillips KM, Ruggio DM, Ashraf-Khorassani M. Phytosterol composition of nuts and seeds commonly consumed in the United States. *J Agric Food Chem.* 2005; 53: 9436-9445

- [20] Rochmi, S.E. and M.S. Sofyan. A diluent containing coconut water, fructose, and chicken egg yolk increases rooster sperm quality at 5°C. *Vet. World*. 2019; 12(7):1116-1120
- [21] Rowe, M. P, Powell, J. G., Kegley, E. B., Lester, T.D.,Williams, C. L., Page, R. J. and Rorie, R. W. Influence of organic versus inorganic trace mineral supplementation on bull semen quality. Available at: <http://arkansasagnews,uark.edu/597-1> (accessed 6 February 2018). 2013.
- [22] Sabudak T. Fatty acid composition of seed and leaf oils of pumpkin. Walnut almond, maize, sunflower and melon. *Chem Nat Compounds*. 2007; 43: 465- 467.
- [23] Shanmugam, M., Vinoth, A., Rajaravindra, K. S. and Rajkumar, U. Thermal manipulation during embryogenesis improves certain semen parameters in layer breeder chicken during hot climatic conditions. *Anim. Reprod. Sci*. 2015; 161, 112-118.
- [24] Stevenson DG, Eller FJ, Wang L, Jane JL, Wang T. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J Agric Food Chem*. 2007; 55: 4005-4013.
- [25] Th'elie, A., A. Bailliard, F. Seigneurin, T. Zerjal, M. Tixier-Boichard, and E. Blesbois. Chicken semen cryopreservation and use for the restoration of rare genetic resources. *Poult. Sci*. 2019; 98:447-455
- [26] Zhang, W.W., L. N Kong, X.Q. Zhang, and Q.B. Luo.(2014) Alteration of HSF3 and HSP70 mRNA Expression In The Tissues Of Two Chicken Breeds During Acute Heat Stress. *Genet. Mol. Res*. 2014; 13: 9787-9794.