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The Effect of Different Natural Extenders in Maintaining The Quality of Sperm Fish (*Cyprinus carpio*)

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Abstract

The high demand for carp as consumed fish nor decorative fish, causes an increasing production. However, an obstacle occurs in production, due to the reproductive characteristics of Common Carp fish. Different times in sperm and egg production from male and female fish cause difficulties in breeding. Hence, it needed sperm preservation so the breeding could be done for the whole year. Natural extenders are the solution to preserve fish sperm without side effects. The purpose of this study was to determine the effect of different types of extenders and different doses on the percentage of sperm motility and viability of carp (*Cyprinus carpio*) after storage This research was conducted at the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya and the Freshwater Cultivation Installation (IBAT Punten), Batu, East Java. The method in this study was Complete Random Design (CRD) with 6 treatments, K (100ml lactate ringer); A (1ml cider + 99ml lactate ringer); B (1ml coconut + 99ml lactate ringer); C (1ml date juice + 99ml lactate ringer); D (1ml honey + 99ml lactate ringer); E (1ml sugar cane + 99ml lactate ringer). The results showed that the highest motility was obtained in the treatment using date extract extender with a motility percentage rate of 77.66%; the highest viability was obtained in the honey extract with 80.71%; and the highest fertility rate was obtained in the honey extract treatment with 72.67 %.

INTRODUCTION

The high demand for *Cyprinus carpio* as fish and as decorative fish from year to year tends to increase, especially in big cities, such as Jakarta, Surabaya, and Bandung (Triyanti and Yulisti, 2012). However, an obstacle occurs in production, due to the reproductive characteristics of Common Carp fish. Different times in sperm and egg production from male and female fish cause difficulties in breeding. In contrast, the motility and viability of sperm will decrease after it is taken out of the

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fish's body. Hence, it needed sperm preservation so the breeding could be done for a whole year (Aliniya *et al.*, 2013).

The purpose of this study was to determine the effect of different types of extenders and different doses on the percentage of sperm motility and viability of carp after storage. Sperm preservation aims to optimize the duration of superior spermatozoa to fertilize fish eggs. Also, to facilitate the type of fish that has different gonad mature stages to breed, and it is also to facilitate transportation for the cement distribution to areas where it is needed According to Gallego and Asturiano (2019), sperm preservation requires good quality and quantity of sperm, while the criteria for good sperm quality for the preservation process are sperm with a motility of more than 70% and a duration of motion of more than two minutes.

According to Cheng *et al.* (2022), the process of preservation of sperm and extender is stored in a refrigerator (4 °C) for 5 days before fertilization. The use of an extender is intended to reduce the activity of spermatozoa, thereby inhibiting energy use and prolonging the life of the spermatozoa (Alavi *et al.*, 2015). The decline in the quality of fish sperm can be suppressed by preservation through dilution and cooling. The addition of a diluent creates suitable conditions for spermatozoa. Preservation of spermatozoa outside the body requires a diluent that can ensure the physical and chemical needs of the spermatozoa so that they can last for a certain period. The energy needed by these spermatozoa is provided by simple sugars (monosaccharides) such as fructose and glucose. The purpose of this study was to determine the effect of different types of extenders and different doses on the percentage of sperm motility and viability of carp after storage.

METHODOLOGY

Ethical Approval

This research was carried out with the ethical code of animal welfare research ethics requirements which include: respect, beneficiary (beneficial), and justice (fair) towards animals; 3Rs principles: Replacement, Reduction, Refinement and 5F/freedom: freedom from hunger and thirst, heat and discomfort, pain, trauma and disease, fear and stress and expressing natural behavior.

Place and Time

This research was conducted at the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, and the Freshwater Cultivation Installation (IBAT Punten), Batu, East Java in Laboratory of Fish Reproduction Fisheries and Marine Sciences, Brawijaya University, June-July 2020.

Research Materials

Tools used in this research were aquarium (40 L), binocular microscope (Olympus CX 23, Philippines), cover glass, object glass, 2 ml Eppendorf tube, 250 ml beaker glass, Hg thermometer, 1 ml syringe, hand tally counter (Heathrow Scientific- HS6594, USA), pool, aerator, 5 ºC refrigerator (FRSS2623AS Frigidaire, USA). The materials used were adult broodstock of *Cyprinus carpio* (The weight ratio of the broodstock used is 2 kg male to 1 kg female (2:1), ringer's lactate, NaCl 100%, eosin, tissue, water, alcohol 70%, coconut water, apple juice, date palm juice, honey, and sugar cane juice.

Research Design

This research used CRD (completely randomized design). Completely Randomized Design is a type of experimental design in which each treatment is randomly assigned to all experimental units.

There are six treatments and three replications in this study as follows: K (100 ml Ringers lactate); A (1% dose of green apple cider (sperm + 1ml of green apple cider with 99 ml of Ringers lactate); B (1% dose of coconut juice (sperm + 1ml coconut juice with 99 ml of Ringers lactate); C (1% dose of date palm juice (sperm + 1ml of date juice with 99 ml of

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Ringers lactate); D (1% dose honey (sperm + 1 ml date juice with 99 ml Ringers lactate); and E (1% dose of sugarcane juice (sperm + 1ml sugarcane juice with 99 ml of Ringers lactate).

The container used for preserving sperm in this study was an Eppendorf tube with a capacity of 2 ml which had been sterilized with 70% alcohol. Then the Eppendorf tube was filled with sperm and natural ingredients in a 1: 9 ratio of Ringer's lactate solution and then stored in the refrigerator at 5ºC for 4 days.

Work Procedure

The stages and procedures in this study were: 1) Broodstock preparation: Sexually mature females are characterized by slow movements, the stomach is enlarged or distended towards the back, if touched it feels soft, the anus is slightly prominent or swollen, and if you massage slowly towards the anus a reddish yellow fluid will come out. For the male parent, the movements are agile, the body is slender, and if you massage it towards the anus, it will release white sperm fluid. 2) Sterilization of experimental containers: Sterilization of the aquarium is done by brushing the surface with liquid soap. The aquarium is then left to dry. After the aquarium looks clean and dry, the next step is to set up the aquarium for the treatment stage.

The container used to store sperm is a 10 ml Eppendorf tube. Sterilization of the tube is done by immersing it in 70% alcohol. The tube is then left to dry from the alcohol. 3) Stripping male broodfish: Male goldfish will release a thick, milky white sperm fluid, while female goldfish will appear swollen on the abdomen towards the urogenital area and when stripped will produce yellow fluid. 4) Observation of spermatozoa parameters: sperm motility, sperm viability (control), and treatment of addition to the concentration of natural material extender. 5) Sample storage: sample storage is carried out using a refrigerator at a temperature of 4°C. 6) Observation of parameters: Fertilization, observation of supporting parameters, water quality.

Data Analysis

Data analysis was performed using descriptive methods. Analysis of variance (ANOVA) and Tukey Test at 5% significant level for Sperm Motility, Sperm Motility, and Sperm Motility.

RESULTS AND DISCUSSION

Sperm Motility

Motility is the ability to move spermatozoa forward in a liquid environment. This movement helps the spermatozoa penetrate the protective cells surrounding the egg. The motility of the spermatozoa of the Carp of the Punten strain was determined by the number of spermatozoa moving from a field of view. In this study, the category of spermatozoa movement was ignored, meaning that all types of spermatozoa movement categories were calculated at the time the percentage level was calculated. Then the motility percentage of carp spermatozoa Punten strain was analyzed statistically using analysis of variance.

Research data on the use of different natural material extenders on the sperm motility of the Carp Punten strain obtained results as presented in Figure 1.

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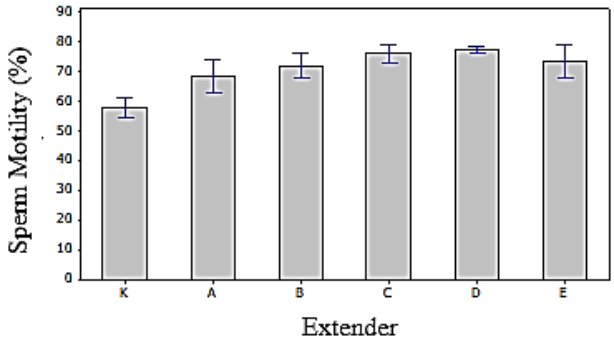

Figure 1. Sperm motility percentage rate of carp (*Cyprinus carpio*) Punten strain.

Based on Figure 1, shows the percentage level (%) of sperm motility of the Punten Carp fish strain with the use of a different natural material extender after motility research was carried out, the best results were obtained in treatment D. In treatment D was a honey extender (1 ml of extract. honey in 99 ml of Ringers lactate) the mean value of motility as shown in the figure is 77.66 ± 0.22%. Following Kusumawati *et al*. (2016) experiment, the higher the movement scale or the percentage level of motility, the better the sperm quality. In comparison, the lowest percentage level was obtained in the control treatment (57.68%).

These results cannot be separated from the condition of the solution in the control treatment, which only consists of Ringer's lactate solution. Maulida *et al*. (2022) state that Ringer's lactate solution does not contain an energy source such as glucose which is needed by spermatozoa to survive. This is in line with the statement of Hayat (2012), basically, in Ringer's solution there is no glucose content which is a substitute energy for fructose in cement plasma which is needed for metabolic activity during storage.

Therefore, the percentage of motility in treatment K was lower than in other treatments. The different superscript letters in the diagram above show significant differences between treatments based on Tukey's test. To determine the effect of the treatment on the motility of the Carp sperm in the Punten strain, an analysis of variance was carried out as presented in Table 1.

Table 1. Sperm Motility variety of the common carp fish (*Cyprinus carpio*) Punten strain.

Source of Variation	Df	SS	MS	F	Sig.
Treatment	5	775.42	155.08	55.93	0.0000
Error	12	33.28	2.77		
Total	17	808.70			

From the results of the calculation of variance above, it can be seen that the Sig value is less than 5%, hence the treatment given has a very significant effect on the motility of the Carp fish (*Cyprinus carpio*) sperm of the Punten strain, so it is continued with the Tukey test and the results presented in Table 2.

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Table 2. Tukey Sperm Motility Test Calculation for Carp (*Cyprinus carpio)* Punten Strain.

Extender	N	SubSet				Notation
		1	2	3	4	
K	3	578.567				A
A	3		685.533			B
B	3		722.933	722.933		Bc
E	3			735.633	735.633	Cd
C	3			762.100	762.100	Cd
D	3				776.600	D

Note: different notations indicate significant differences between treatments.

Based on the Tukey test, it was found that the K treatment gave different motility percentage results from the others, with the smallest motility percentage level. It proves that without using an extender that can provide energy for the sperm, sperm motility will not be as good as the sperm with a natural extender.

Treatments A and B were significantly different. Treatment B was not significantly different from treatment E and C, then treatment E was not significantly different from C and D. The treatment with the highest percentage level of motility was treatment D, with 1 ml honey extract in 99 ml Ringers lactate which resulted in 77.66%. According to Srivastava and Pande (2017), individual motility can be assessed from sperm motion, if progressive movement or active forward motion is the best motion and is considered to be 70% greater.

Research by Nainggolan *et al*. (2015) also showed that giving honey extender can increase the sperm motility of fish because honey contains fructose which can provide energy to sperm during the preservation process. According to Pojprasath *et al*. (2011), sperm preservation requires fructose which is used as a source of energy for spermatozoa and can reduce the rate at which spermatozoa permeability breaks, so that the need for energy and nutrients in the form of ATP is not inhibited and spermatozoa can last longer.

Fructose can be used to produce ATP in both anaerobic and aerobic conditions. It means that if fructose is metabolized under anaerobic conditions it will produce 2 ATP or 1,400 calories, while in aerobic conditions, fructose metabolism is 9 times more efficient in producing energy. The total energy of 38 ATP is 266,000 calories. When there is sufficient oxygen, the fructose molecules are completely metabolized into carbon dioxide and water (Hidayaturrahmah, 2017). In this case, honey is a simple type of carbohydrate, which consists of 38.5% fructose and 31% glucose (Gündoğdu *et al*., 2019). According to Sihombing (1997), honey is a source of sugar which can also be used as a source of nutrients containing various types of sugar, including 41% fructose, 35% glucose, and 1.9% sucrose. Honey has a high sugar content in the form of fructose 38.19%, glucose 31%, and sucrose 1.31%.

Sperm Viability

The percentage of viability is an indicator to determine the quality of spermatozoa and determine how many spermatozoa are alive (viable) or not alive (unviable) which in the appearance of immobile or immotile spermatozoa and turns to pink color when given eosin dye (Ludwig and Frick, 2012). The difference between living and dead sperm can be seen in Figure 2.

The research data on the use of different natural material extenders on the viability of sperm in the Punten strain of Carp are shown in Table 3.

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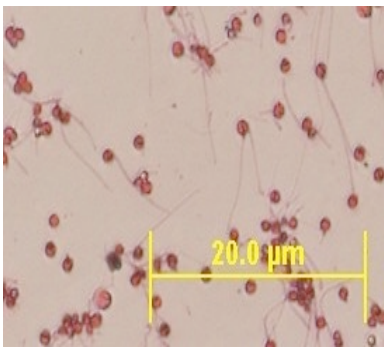

Figure 2. Difference between live and dead sperm.

Table 3. Data on the use of different natural material extenders on the viability of sperm in the Punten strain of Carp fish (*Cyprinus carpio*).

Treatment	Repetition			Total	Mean
	1	2	3		
K	63.12	60.27	62.90	186.29	62.01 ± 2.51
A	78.58	73.83	75.44	227.85	75.95 ± 5.84
B	63.12	60.27	62.90	186.29	62.01 ± 2.51
C	79.85	78.49	74.86	233.21	77.73 ± 6.65
D	80.05	81.98	80.09	242.12	80.71 ± 1.22
E	76.33	77.52	77.52	231.37	77.12 ± 0.47

The results obtained from Table 3 can be shown as follows using a different natural material extender, the lowest percentage of viability was obtained in treatment K (100 ml of Ringers lactate without giving fruit juice) and treatment B. While the highest percentage was obtained in the honey extract treatment with a percentage level. viability of 80.71%, which was then followed by treatment C (77.73%), E (77.12%), and A (75.59%).

Based on Table 3 above, it can be shown that the viability of the Carp fish sperm of the Punten strain with the use of different natural material extenders obtained the best results for the percentage level of viability in treatment D. Where the D treatment is honey extract extender (1 ml of honey extract in 99 ml of Ringer's lactate) which obtained a mean percentage of viability of 80.71 ± 1.22%. Treatment using a honey extender got the highest result because of the addition of glucose and fructose in the sperm dilution. The length of time for sperm activity becomes longer so that the sperm has a lot of time to find the egg. This condition is following El-Sheshtawy *et al*. (2016).

According to Bustani and Baiee (2021), the addition of a honey extender solution as a diluent can sustain the life of spermatozoa by providing nutrients for energy sources (glucose and fructose). In the preservation process of spermatozoa, a diluent is needed that is not only used as a sperm thinner but also has to be able to function as a source of nutrition for the spermatozoa so that the spermatozoa can be preserved. The use of sperm thinners must be able to maintain the viability of spermatozoa before use in time.

Requirements for the diluent are to be able to provide nutrition for spermatozoa needs during storage, allow spermatozoa to move actively, be non-toxic, and protect spermatozoa from cold shock when the freezing process could damage the cell plasma membrane which results in spermatozoa death (Fadhillah *et al*., 2022). According to Toelihere (2004), spermatozoa prefer simpler atomic groups in the form of glucose and fructose before further hydrolysis of sucrose is used as energy for movement. Furthermore, Pamungkas *et al*. (2020) explained that the metabolic process of spermatozoa can take place well in a diluent containing

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degraded sugars such as glucose and fructose. To determine the effect of the treatment on the viability of the Carp fish sperm of the Punten strain, an analysis of variance was carried out as presented in Table 4.

Table 4. Sperm viability variability of Carp (*Cyprinus carpio*) Punten strain.

Source of Variation	Df	SS	MS	F	Sig
Treatment	5	1033.17	206.634	64.547	0.00
Error	12	38.42	3.201		
Total	17	1071.59			

The results of the calculation of variance above can be explained that the Sig value is less than 5%, so it can be concluded that the treatment given has a very significant effect on the viability of the Carp fish sperm of the Punten strain, so that it is continued with the Tukey test and the results, presented in Table 5.

Table 5. Tukey test result of sperm viability for Carp (*Cyprinus carpio*) Punten strain.

Extender	N	Subset		Notation
		1	2	
C	3	62.097		A
K	3	62.97		A
A	3		75.950	B
E	3		77.123	B
B	3		77.123	B
D	3		80.707	B

Note: different notations indicate significant differences between treatments.

Based on the Tukey test, it was found that control (K) gave a percentage of viability that was not significantly different from treatment C, while treatments A, E, B, and D gave results that were not significantly different from one another. Treatment C was significantly different from treatments A, E, B, and D. The treatment with the highest level of viability was treatment D, with 1 ml of honey extract in 99 ml of Ringers lactate which resulted in viability of 80.71%.

According to Laughlin (2014), apart from containing fructose which can be directly absorbed by the blood so that it can quickly produce energy, honey also has antibacterial properties that can keep sperm in good condition until the fertilization process. Honey is a sweet liquid derived from plant nectar which is processed by worker bees into honey and stored in honeycomb cells (Zaheen *et al*., 2020). Various advantages of honey as a high-nutrient food have been known since ancient times. Honey contains vitamins A, B1, B2, B3, B5, B6, C, D, E, and K, beta carotene, flavonoids, phenolic acid, uric acid and nicotinic acid. According to Fujaya (2008), the lifespan of spermatozoa that have been removed from the testis is highly dependent on the energy supply contained in the sperm's body. This is reinforced by a statement from Hidayaturrahmah (2017).

Sperm Fertility

The fertility rate is the ability of the fish's sperm to fertilize an egg. Self-fertilization or fertilization is the meeting between spermatozoa and egg cells. The fertilization process in an egg is strongly influenced by egg quality, sperm quality, and sperm speed to move spontaneously so that it can enter the microphile hole in the egg. In the fertilization process, the spermatozoa nucleus combines with the egg nucleus in the cytoplasm to form a zygote (Faqih, 2011). Fertilization of fish eggs is supported by the presence of a

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substance called fertilizin which stimulates spermatozoa to pursue eggs released by the female parent. The fertilizers are released by the egg at the last moment when the egg is released and is ready to be fertilized. Fertilization of fish eggs can be said to occur when spermatozoa enter the eggs through microphils (Murtidjo, 2001).

Research data using different natural material extender on the fertility of Carp fish sperm Punten strain obtained the best fertilization results, namely in treatment C with an average fertilization rate of 78.00% and the lowest fertilization result in the control treatment with an average amounted to 42.67%. The egg fertilization rate of the carp Punten strain can be seen in Figure 3.

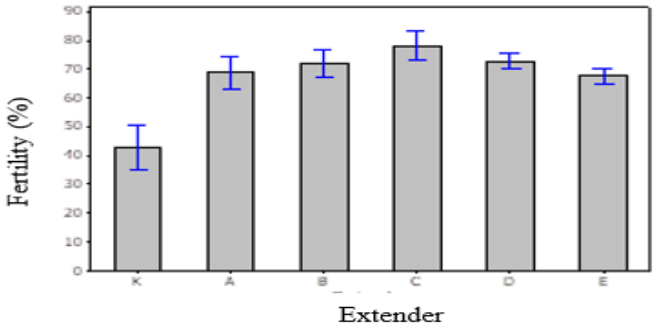

Figure 3. Punten strain carp sperm fertility.

Based on the Tukey test, it was found that treatment K gave a percentage of fertility that was significantly different from other treatments, while treatments E, A, B, and D gave a percentage of fertility that was not significantly different. Treatments D and C were not significantly different. Treatment C was significantly different from treatments K, A, B, and E. The treatment with the highest fertility rate was treatment C, with 1 ml of date juice in 99 ml of Ringers lactate which resulted in a fertility of 78.00%. Based on research conducted by Félix *et al*. (2020), dates contain 68% glucose and 8.4 mg of vitamin C in 12 g of date juice which acts as an antioxidant.

Glucose is the source of energy for sperm, while antioxidants are believed to increase sperm fertility in spawning. and discussion includes data on research results and their discussion. Research results can be listed in the form of schematics, tables, pictures, or photos during the implementation of the research. The presentation of data in the form of an average value should also include the standard deviation or standard error.

CONCLUSION

Treatment D with the use of 1 ml honey extender in 99 ml of Ringers lactate gave significant results on the motility and viability of Carp sperm, namely 77.66% and 80.71%, respectively. Meanwhile, treatment C using a date extender of 1 ml in 99 ml of Ringers lactate can increase the fertility of Carp sperm by 78%, 6% greater than using a honey extender. Both treatments (honey and dates) give the best results, because they have a high glucose content as a source of energy for Carp sperm, and contain antibiotics and antioxidants that can increase the ability of sperm to fertilize. The conclusion describes the results of the interpretation of the discussion that is formulated briefly, concisely, and clearly to provide a brief description of the research results or prove the truth of the hypothesis.

CONFLICT OF INTEREST

The author in this case declares that there is no conflict of interest between all authors when writing and publishing the manuscript. In the process of writing this paper, all authors worked together to

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divide their roles from the research stage, and data processing, to writing this paper.

AUTHOR CONTRIBUTION

The writing team for this paper includes Mr. Abd. Rahem Faqih as the main author and head of research, Mrs. Febri Eka Supriatin as, Mrs. Aulia Rahmawati, Mrs. Septi Anitasari as a research assistant in writing and processing data, Mr. Gilang Drajat Maulana as a technical research assistant, and Mr. Alim as a proofreader.

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REFERENCES

Alavi, S.M.H., Ciereszko, A., Hatef, A., Křišťan, J., Dzyuba, B., Boryshpolets, S., Rodhina, M., Cosson, J. and Linhart, O., 2015. Sperm morphology, physiology, motility, and cryopreservation in Percidae. In *Biology and Culture of Percid Fishes: Principles and Practices*, pp.163-191. https://doi.org/10.1007/978-94-017-7227-3_5

Aliniya, M., Khara, H., Noveiri, S.B. and Dadras, H., 2013. Influence of age of common carp (Cyprinus carpio) broodstock on reproductive traits and fertilization. *Turkish Journal of Fisheries and Aquatic Sciences*, *13*(1), pp.19-25. https://doi.org/10.4194/1303-2712-v13_1_03

Bustani, G.S. and Baiee, F.H., 2021. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Veterinary World*, *14*(5), pp.1220-1233. https://doi.org/10.14202/vetworld.2021.1220-1233

Cheng, Y., Zhang, S., Linhartová, Z., Shazada, N.E. and Linhart, O., 2022. Common carp (*Cyprinus carpio*) sperm reduction during short-term *in vitro* storage at 4° C. *Animal Reproduction Science*, *243*, 107017. https://doi.org/10.1016/j.anireprosci.2022.107017

El-Sheshtawy, R.I., El-Badry, D.A., El-Sisy, G.A., El-Nattat, W.S. and Almaaty, A.M.A., 2016. Natural honey as a cryoprotectant to improve Arab stallion post-thawing sperm parameters. *Asian Pacific Journal of Reproduction*, *5*(4), pp.331-334. https://doi.org/10.1016/j.apjr.2016.06.004

Fadhillah, Pamungkas, M.A.B., Kristanto, A.H. and Lestari, R. and Abinawanto, 2022. The Effects of Brown Sugar as a Natural Cryoprotectant on Tor Soro (Valenciennes 1842) Spermatozoa Quality. *Journal of Hunan University Natural Sciences*, *49*(3), pp.1-13. https://doi.org/10.55463/issn.1674-2974.49.3.1

Faqih, A.R., 2011. Penurunan motilitas dan daya fertilitas sperma ikan lele dumbo (Clarias spp) pasca perlakuan stress kejutan listrik. *The Journal of Experimental Life Science*, *1*(2), pp.72-82. https://doi.org/10.21776/ub.jels.2011.001.02.03

Félix, F., Oliveira, C.C.V. and Cabrita, E., 2020. Antioxidants in fish sperm and the potential role of melatonin. *Antioxidants*, *10*(1), 36. https://doi.org/10.3390/antiox10010036

Fujaya, Y., 2008. Fisiologis Ikan: Dasar Pengembangan Teknik Perikanan. Penerbit Rineka Cipta. Jakarta. Jakarta. p.179.

Gallego, V. and Asturiano, J.F., 2019. Fish sperm motility assessment as a tool for aquaculture research: a historical

Cite this document as Faqih, A.R., Supriatin, F.E., Rahmawati, A., Anitasari, S,. Maulana, G.D. and Alim, M.B., 2024. The Application of Different The Effect of Different Natural Extenders in Maintaining The Quality of Sperm Fish (*Cyprinus carpio*). *Journal of Aquaculture and Fish Health*, *13*(1), pp.80-89.

approach. *Reviews in Aquaculture*, *11*(3), pp.697-724. https://doi.org/10.1111/raq.12253

Gündoğdu, E., Çakmakçı, S.and Şat, İ.G., 2019. An overview of honey: its composition, nutritional and functional properties. *Journal of Food Science and Engineering*, *9*, pp.10-14. https://doi.org/10.17265/2159-5828/2019.01.003

Hayat, M.A., 2012. *Fixation for electron microscopy*. Elsevier. p.473.

Hidayaturrahmah, 2017. Waktu motilitas dan viabilitas spermatozoa ikan mas (Cyprinus carpio L) pada beberapa konsentrasi larutan fruktosa. *BIOSCIENTIAE, 4(1)*, pp.124–135. https://doi.org/10.20527/b.v4i1.158

Laughlin, M.R., 2014. Normal roles for dietary fructose in carbohydrate metabolism. *Nutrients*, *6*(8), pp.3117-3129. https://doi.org/10.3390/nu6083117

Ludwig, G.and Frick, J., 2012. *Spermatology: atlas and manual*. Springer Science & Business Media. Salzburg.

Maulida, S., Eriani, K., Fadli, N., Nur, F.M., Handayani, L.S.and Muchlisin, Z.A., 2022. The effect of extender on the quality of climbing perch (Anabas testudineus) sperm after refrigerated storage. *Aquaculture Research*, *53*(12), pp.4497-4504. https://doi.org/10.1111/are.15947

Murtidjo, B.A., 2001. Beberapa metode pembenihan ikan air tawar. Penerbit Kanisius. Yogyakarta.

Nainggolan, R., Monijung, R.D. and Mingkid, W., 2015. Penambahan madu dalam pengenceran sperma untuk motilitas spermatozoa fertilisasi dan daya tetas telur ikan nila (*Orechromis niloticus*). *Jurnal Budidaya Perairan. 3*(1), pp.131-140. https://doi.org/10.35800/bdp.3.1.2015.6948

Pamungkas, M.A.B., Abinawanto, Arifin, O.Z. and Kristanto, A.H., 2020. The spermatozoa viability of kancra fish (*Tor soro*, Valenciennes 1842) 48-hour after freezing: effect of brown sugar as natural cryoprotectant. *IOP Conference Series: Earth and Environmental Science*, *441*, 012063. https://doi.org/10.1088/1755-1315/441/1/012063

Pojprasath, T., Lohachit, C., Techakumphu, M., Stout, T. and Tharasanit, T., 2011. Improved cryopreservability of stallion sperm using a sorbitol-based freezing extender. *Theriogenology*, *75*(9), pp.1742-1749. https://doi.org/10.1016/j.theriogenology.2011.01.014

Sihombing, D.T.H., 1997. Ilmu Ternak Lebah Madu. Yogyakarta: Gadjah Mada University Press. p.243.

Srivastava, N. and Pande, M., 2017. Estimates of Sperm Motility. In *Protocols in Semen Biology (Comparing Assays)*, pp.43-55. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-10-5200-2

Toelihere, M.R., 2004. Inseminasi Buatan Pada Ternak. Angkasa, Bandung, Jawa Barat. p.292.

Triyanti, R. and Yulisti, M., 2012. Rantai Pemasaran Ikan Koi (Cyprinus carpio) di Kabupaten Blitar, Jawa Timur. *Buletin Ilmiah Marina Sosial Ekonomi Kelautan dan Perikanan*, *7*(1), pp.14-20. http://dx.doi.org/10.15578/marina.v7i1.4593

Zaheen, Z., Yatoo, A.M., Ali, S., Ali, M.N., Majid, S., Rasool, S., Rashid, S.M., Ahmad S.B., Mir, M.U.R. and Zehra, U., 2020. Honey: types, composition, and antimicrobial mechanisms. In *Therapeutic Applications of Honey and its Phytochemicals: Vol. 1*, pp.193-214. https://doi.org/10.1007/978-981-15-6799-5

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