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Effect of Addition of Glucose or Fructose in Three Types of Crystalloid Fluid on Spermatozoa Quality of Gaga' Chicken from South Sulawesi, Indonesia

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Khaeruddin presented the concept. SW and Khaeruddin peformed visualization and wrote, reviewed and edited the manuscript. GC, Khaeruddin, MY, SW and Sahiruddin planned methodology. Khaeruddin, Hermawansyah and Sahiruddin performed formal analysis.

Key words
Gaga chicken, Sperm, Diluents, Crystalloid fluids, Sugar

ABSTRACT

Gaga's chicken is a genetic resource of Indonesian livestock which must be protected and preserved. The increase in the gaga chicken population can be made by artificial insemination techniques and diluents are important factors in determining the quality of chicken spermatozoa. The aim of this study was to determine the effect of the use of three types of crystallized liquids and two types of monosaccharides on the quality of Gaga chicken spermatozoa. This study used a completely randomized design with a factorial pattern with the first factor being the type of crystalloid liquid (Ringer's lactate, Ringer's acetate, and KA-EN 3B) supplemented with egg yolk and the second factor being the addition of sugar (control, glucose, and fructose). The semen was diluted at a ratio of 1:10 and stored at 5 °C for 48 h. The results showed that the type of diluent treatment affected abnormal morphology, progressive motility, local motility, total motility, immotil, linearity and straightness. In general, Ringer's acetate-yolk glucose diluent is better in maintaining the quality of Gaga's chicken spermatozoa during storage.

INTRODUCTION

Indonesia has various types of native chickens which require serious attention to preserve and improve the genetic quality. Ketawa, also known as Gaga, is an example of a native chicken from South Sulawesi Province, Indonesia, and is popular among hobbyists due to its unique voice, which is slow and dangdut-like (Bugiwati and Ashari, 2013). Based on the Minister of Agriculture decree No. 2920/Kpts/OT.140/6/2011, the Gaga chicken is a wealth of Indonesian livestock genetic resources that need to be protected and preserved. Therefore, there is a need to increase its population and preservation as the original Indonesian chicken germplasm.

The population of Gaga chickens is increased by improving maintenance management, including mating using artificial insemination which increases the population of poultry rapidly compared to the natural type because males are used to mate with more females. Meanwhile, diluents are used to raise the volume of semen, thereby increasing the mating ratio. The diluent must contain similar components in seminal plasma, be isotonic, non-toxic and able to maintain the quality of spermatozoa during storage. Therefore, crystalloid liquids are practically used as a chicken semen diluent because it is isotonic and easily available at the nearest pharmacy.

A crystalloid fluid is an aqueous solution of mineral salts and other small, water-soluble molecules. Most commercially available crystalloid solutions are isotonic to human plasma, this means that the fluids correspond to the concentrations of various solutes present in the plasma (Epstein and Waseem, 2021). Some examples that are commercially available in Indonesia include ringers lactate, ringers acetate, and KA-EN 3B® which contain minerals

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such as sodium and potassium chloride. Zhaowang and Mengzhou (2008) stated that inorganic salts namely sodium chloride and potassium chloride were, respectively used under the osmotic pressure of 350 mmol/L, consequently, the survival time and index of sperms were both higher. The use of ringers lactate as a diluent has been intensively applied to various studies on local chickens in Indonesia (Telnoni *et al*., 2021; Khaeruddin and Hastuti, 2020; Khaeruddin and Amir, 2019; Iswati *et al*., 2018; Telnoni *et al*., 2017; Junaedi *et al*., 2017). However, other crystalloid liquids such as ringer's acetate and KA-EN 3B® have not been tested as a semen diluent, even though this liquid has a greater potential compared to ringer's lactate.

The presence of simple sugars in a diluent is needed as an energy source for spermatozoa. Meanwhile, glucose and fructose have been extensively studied in mammalian semen as an energy source. The addition of glucose increased motion characteristics of stallion sperm during long-term storage (5 days) (Hernández-Avilés *et al*., 2021), while spermatozoa obtained from bull epididymis and cryopreserved use fructose as an optimal energy source to reduce the loss of viability and motility (Pappa *et al*., 2019). Furthermore, the single composition extenders of glucose, sucrose, and fructose all function appropriately to protect the integrity of chicken sperms (Zhaowang and Mengzhou, 2008). Data on the addition of sugars to diluents and the effect on the quality of chicken spermatozoa is still lacking. Therefore, this study aims to determine the effect of using three types of crystalloid liquids and two types of monosaccharides on the quality of Gaga chicken spermatozoa at cooled storage.

MATERIALS AND METHODS

Collection and evaluation of the fresh semen

Five Gaga chickens aged approximately 10 months old were used in this study after being kept in individual cages measuring 40 x 50 x 70 cm^3, and fed daily with 150 grams as well as water ad libitum. Semen collection was performed in the morning from 7:30 am using a massage technique based on Stelzer *et al*. (2005). Semen was obtained by placing the left hand on the back of the chicken and massaging around the cloaca with two fingers until the papilla protruded. This protrusion of the papilla was followed by the ejaculation of fresh semen. The semen was collected using a tuberculin syringe and brought to the laboratory for macroscopic (volume, color, viscosity, and pH) and microscopic evaluation.

Sperm concentration was calculated in the Neubaeur chamber under a microscope with a magnification of 160 x (Boeco, Germany). The mass movement was evaluated with a fresh semen drop object observed under a microscope with a magnification of 160 x. evaluation of individual motility was carried out subjectively on diluted semen under a microscope magnification of 640x. Eosin-nigrosine stain (Merck, KgaA, Darmstadt Germany) was used to evaluate the viability and the percentage of abnormal morphology. A drop of semen was mixed with a drop of eosin-nigrosine then rubbed on a glass slide and then warmed on a heating table for 5 seconds. Approximately 200 spermatozoa were observed with a microscope which has 640x magnification for viability calculation by counting the color and non-color-absorbing samples, followed by calculating the abnormal morphology of viable spermatozoa.

Diluent preparation

Three types of crystalloid solutions were used as basic diluents, namely ringer's lactate, ringer's acetate, and Ka-EN 3B®. Ringer's lactate (PT. Widatra Bakti, Indonesia) contained 1.55 g of sodium lactate, 3 g of sodium chloride, 0.15 g potassium chloride, and 0.1 g of calcium chloride in 500 mL sterile water, with osmolarity 274 mOsm L^{-1}. KA-EN 3B® (PT. Otsuka, Indonesia) which contained anhydrous dextrose 13.5 g, sodium chloride 0.875 g, potassium chloride 0.75 g, sodium lactate 1.1 g in 500 mL sterile water, with osmolarity 290 mOsm L^{-1}. Ringer's acetate (Asering®, PT. Otsuka, Indonesia) contained 1.9 g sodium acetate, 3 g sodium chloride, 0.15 potassium chloride, and 0.1 g calcium chloride in 500 mL sterile water, with osmolarity 273 mOsm L^{-1}. The basic diluent was added with 9% chicken egg yolk and centrifuged at 2000 rpm for 30 minutes, then the supernatant was collected and added with penicillin 1000 IU mL^{-1} (PT. Meiji, Indonesia) and 1 mg mL^{-1} streptomycin (PT. Meiji, Indonesia).

Nine tubes were filled with different extenders. The first tube was filled with ringer's lactate + egg yolk (RLY), the second tube was filled with RLY + glucose 2% (w/v) (Merck, KgaA, Darmstadt Germany), the third tube was filled with RLY + fructose 2% (Merck, KgaA, Darmstadt Germany), the fourth tube was filled with ringer's acetate + egg yolk (RAY), the fifth tube was filled with RAY + glucose 2% (w/v), the sixth tube was filled with RAY + fructose 2% (w/v), the seventh tube was filled with KA-EN 3B® + egg yolk (K3Y), the eighth tube was filled with K3Y + glucose 2% (w/v), and the ninth tube was filled with with K3Y + fructose 2% (w/v).

Dilution, storage and evaluation

The semen was diluted in a ratio of 1:10, stored at 5°C for 48 h, and then evaluated for viability and abnormal morphology of the spermatozoa by eosin-nigrosine staining. Furthermore, the motility and kinematic parameters were

monitored using a computer-assisted sperm analysis system (CASA, Sperm Vision, Minitube, Tiefenbach, Germany), connected to a trinocular light microscope (Zeiss AXIO Scope A1, US). The sperm motility parameters include total motility, progressive motility, local motility and immotile. Sperm kinematic parameters inclue distance curve-line (DCL), distance straight-line (DSL) dan distance average path (DAP), velocity curve linear (VCL), velocity average pathway (VAP), velocity straight line (VSL), linearity (LIN), straightness (STR), wobble (WOB), beat cross frequency (BCF), amplitude lateral head (ALH) dan average orientation dan change of head (AOC).

Statistical analysis

Statistical analysis for multiple comparisons was performed using a completely randomized design with factorial pattern with nine treatment combinations and five replications. viability, abnormal morphology, motility parameters and kinematic parameters after storage were analyzed using ANOVA, if the f-value is significant ($p < 0.05$) then it was followed by duncan multiple range test. statistical analysis used spss 16 applications on windows.

RESULTS

Characteristics of fresh semen

The fresh semen examination results showed that the volume was relatively low, namely 0.18 ml with milky color, and a pH of 7.54. The concentration of spermatozoa was relatively low, namely 1.937 billion ml^{-1}, motility 78%, viability 99.13%, and abnormal morphology of 7.44% (Table I).

Table I. The average of fresh semen characteristics of Gaga chicken.

Parameter	Mean ± SEM
Volume (ml)	0.18±0.05
Color	Milky
Consistency	Thick
pH	7.54±0.42
Sperm concentration (x10^6/mL)	1937±230.14
Movement of sperm mass	++
Motility (%)	78±2.00
Viability (%)	99.13±0.5
Abnormal morphology (%)	7.44±0.87

Sperm viability after storage

Moreover, the viability of spermatozoa after 24 and 48 h storage did not differ between the treatments ($P>0.05$) as indicated by values ranging from 96.78-98.72% and 95.87-97.76%, respectively (Table II).

Table II. Viability of Gaga chicken sperm at 24 and 48 h of cold storage that dilution with with ringer lactate-yolk, ringer acetate-yolk, and KA-EN 3B-yolk added with glucose or fructose.

Semen sample	Viability (%)	
	24 h storage	48 h storage
Ringer lactate-yolk		
No sugar	97.60	96.80
Glucose	98.72	97.20
Fructose	97.61	97.20
Ringer acetate-yolk		
No sugar	97.22	96.79
Glucose	97.02	96.92
Fructose	97.86	97.76
KA-EN 3B-yolk		
No Sugar	97.33	97.29
Glucose	96.78	95.87
Fructose	97.88	95.98
SEM	0.208	0.305
Effects		
Crystaloid extender	0.374	0.531
Sugar	0.724	0.893
Extender x sugar	0.417	0.725

Sperm abnormal morphology after storage

The diluent treatment significantly affected ($P<0.05$) the abnormal morphology of spermatozoa at 24 and 48-h storage (Table III), as well as the interaction between the type of crystalloid diluent and the type of sugar. The use of KA-EN 3B®-yolk diluent produced lower abnormal morphology compared to ringer's acetate-yolk and ringer's lactate-yolk diluents, while the addition of glucose and fructose generally reduced the abnormal morphology of the sperm. At 24 h of storage, the lowest morphological abnormalities (11.39-18.65%) were found in the treatment with ringer's lactate-yolk glucose, ringers acetate-yolk glucose, ringer's lactate-yolk fructose, KA-EN 3B®-yolk diluent, while at 48 h, abnormal morphology increased with the lowest number ranging from 16.70-22.87% in ringer'sa acetate-yolk glucose, ringer'sa acetate-yolk fructose, and KA-EN 3B®-yolk diluent with or without sugar.

Sperm motility after storage

The type of sugar treatment had a significant effect ($P<0.05$) on all parameters of sperm motility except local motility, while the type of diluent crystalloid had no effect ($P>0.05$) (Table IV). The interaction between the type of crystalloid diluent and sugar affected ($P<0.05$) on all motility parameters. The highest total motility was found in the ringer acetate-yolk with glucose (76.18%), while the lowest (45.58-54.38%) was found in the ringers lactate-yolk diluent without sugar, and KA-EN 3B-yolk glucose.

Table III. Abnormal morphology of Gaga chicken sperm at 24 and 48 h of cold storage that dilution with ringer lactate-yolk, ringer acetate-yolk, and KA-EN 3B-yolk added with glucose or fructose.

Semen sample	Abnormal morphology (%)	
	24 h storage	48 h storage
Ringer lactate-yolk		
No sugar	47.07^{a}	61.71^{a}
Glucose	18.65^{c}	28.99^{cd}
Fructose	28.05^{b}	31.13^{c}
Ringer acetate-yolk		
No sugar	28.55^{b}	38.54^{b}
Glucose	11.39^{e}	17.25^{e}
Fructose	12.92^{c}	21.28^{e}
KA-EN 3B-yolk		
No sugar	17.54^{c}	22.87^{de}
Glucose	11.96^{e}	16.70^{e}
Fructose	16.17^{c}	19.94^{e}
SEM	0.811	0.848
Effects		
Crystaloid extender	0.000	0.000
Sugar	0.000	0.000
Extender x sugar	0.000	0.000

Note: Different superscripts in the same column show significant differences (P <0.05).

Table IV. Motility parameters of Gaga chicken sperm at 48 h of cold storage that dilution with with ringer lactate-yolk, ringer acetate-yolk, and KA-EN 3B-yolk added with glucose or fructose.

Semen sample	Variable of motility (%)			
	Total motility	Progressive	Local	Immotil
Ringer lactate-yolk				
No sugar	53.60^{ab}	33.62^{ab}	20.00^{abc}	46.40^{de}
Glucose	63.52^{bcd}	34.40^{ab}	29.08^{d}	36.48^{bcd}
Fructose	59.98^{bcd}	40.50^{abcd}	19.48^{abc}	40.02^{bcd}
Ringer acetate-yolk				
No sugar	45.58^{a}	30.88^{a}	14.68^{a}	54.42^{e}
Glucose	76.18^{e}	52.52^{d}	23.64^{bcd}	23.82^{a}
Fructose	66.26^{d}	47.16^{cd}	19.10^{ab}	33.76^{b}
KA-EN 3B-yolk				
No Sugar	64.96^{cd}	39.30^{abc}	25.66^{cd}	35.04^{bc}
Glucose	54.38^{abc}	36.84^{abc}	17.54^{ab}	45.62^{cde}
Fructose	64.76^{cd}	44.52^{bcd}	20.26^{abc}	35.24^{bc}
SEM	1.153	1.313	0.673	1.153
Effects				
Crystaloid extender	0.435	0.087	0.93	0.436
Sugar	0.002	0.017	0.55	0.002
Extender x sugar	0.000	0.042	0.00	0.000

Note: Different superscripts in the same column show significant differences (P <0.05).

Furthermore, the highest progressive motility (40.5-52.52%) was observed in the treatment with ringers lactate-yolk fructose, KA-EN 3B-yolk fructose, ringers acetate-yolk fructose and ringers acetate-yolk glucose, while the lowest immotile was found in ringers acetate-yolk glucose and the highest in KA-EN 3B-yolk fructose and ringer acetate-yolk without sugar.

Kinematic parameters after storage

The results showed that the type of crystalloid diluent and sugar interactions did not affect the DCL (30.3-37.37 µm), DAP (16.68-18.58 µm), DSL (10.93-12.84 µm), VCL (74-93.63 µm s^{-1}), VAP (41.19-46.85 µm s^{-1}), VSL (27.6-32.1 µm s^{-1}), WOB (49.2-58.4 %), BCF (19.79-22.98 Hz), ALH (5.1-5.53 µm), dan AOC (14.48-17.86) (Tables V and VI). However, the type of sugar treatment affected LIN and STR, for example, the addition of glucose to the KA-EN 3B-yolk diluent decreased sperm linearity. The highest linearity ranged from 35.2-43.4% while the lowest ranged from 31-37.88%. Moreover, the addition of glucose to ringer's lactate-yolk and KA-EN 3B-yolk decreased straightness, while the addition of fructose had no effect on each type of crystalloid diluent. The highest straightness ranged from 71.2-77.8%, while the lowest ranged from 63.2-70.4%.

Table V. DCL, DAP, DSL, VCL, VAP and VSL of Gaga chicken sperm at 48 h of cold storage that dilution with with ringer lactate-yolk, ringer acetate-yolk, and KA-EN 3B-yolk added with glucose or fructose.

Semen sample	DCL (µm)	DAP (µm)	DSL (µm)	VCL (µm s^{-1})	VAP (µm s^{-1})	VSL (µm s^{-1})
Ringer lactate-yolk						
No sugar	30.30	16.68	12.84	74.00	41.19	32.10
Glucose	34.33	18.53	12.28	82.61	45.17	30.27
Fructose	35.53	17.40	12.13	90.31	44.87	31.73
Ringer acetate-yolk						
No sugar	32.43	17.09	11.99	83.03	44.40	31.36
Glucose	35.41	18.03	12.15	87.22	44.77	30.31
Fructose	37.37	18.58	12.35	93.63	46.85	31.23
KA-EN 3B-yolk						
No Sugar	30.96	17.24	12.18	74.90	42.07	29.95
Glucose	37.11	17.61	10.93	91.43	43.96	27.60
Fructose	33.31	18.06	12.66	81.90	44.92	31.53
SEM	0.890	0.364	0.191	2.225	0.913	0.544
Effects						
Extender	0.726	0.916	0.580	0.520	0.700	0.432
Sugar	0.090	0.419	0.385	0.093	0.400	0.254
Extender x sugar	0.844	0.921	0.434	0.683	0.114	0.902

DCL, distance curve-line; DAP, distance average path; DSL, distance straight-line; VCL, velocity curve linear; VAP, velocity average pathway; VSL, velocity straight line.

Table VI. LIN, STR, WOB, BCF, ALH and AOC of Gaga chicken sperm at 48 h of cold storage that dilution with with ringer lactate-yolk, ringer acetate-yolk, and KA-EN 3B-yolk added with glucose or fructose.

Semen sample	LIN (%)	STR (%)	WOB (%)	BCF (Hz)	ALH (µm)	AOC
Ringer lactate-yolk						
No sugar	43.40^{b}	77.80^{c}	56.00	22.20	5.53	15.21
Glucose	36.80^{ab}	67.80^{ab}	54.60	21.00	5.19	15.24
Fructose	36.00^{ab}	71.20^{bc}	50.20	21.31	5.10	17.86
Ringer acetate-yolk						
No sugar	37.80^{ab}	70.40^{ab}	53.60	22.98	5.39	14.88
Glucose	35.20^{ab}	68.20^{ab}	51.40	21.50	5.29	16.40
Fructose	33.40^{a}	66.80^{ab}	50.00	21.03	5.53	16.75
KA-EN 3B-yolk						
No Sugar	42.60^{b}	71.60^{bc}	58.40	21.67	5.18	16.11
Glucose	31.00^{a}	63.20^{a}	49.20	19.79	5.42	15.73
Fructose	38.80^{ab}	70.40^{ab}	55.40	20.74	5.46	14.48
SEM	0.839	0.754	0.804	0.348	0.102	0.307
Effects						
Extender	0.289	0.069	0.386	0.422	0.878	0.636
Sugar	0.005	0.003	0.061	0.176	0.953	0.442
Extender x sugar	0.265	0.255	0.239	0.962	0.775	0.121

Note: Different superscripts in the same column show significant differences (P <0.05). LIN, linearity; STR, straightness; WOB, wobble; BCF, beat cross frequency; ALH, amplitude lateral head; AOC, average orientation dan change of head.

DISCUSSION

The use of crystalloid and sugar diluents had no effect on the viability of spermatozoa stored for 24 and 48 h, indicating that both fructose and glucose are suitable for maintaining the life of spermatozoa. A previous study on ram spermatozoa reported that the use of glucose and fructose had no significant effect on viability (Panyaboriban *et al*., 2015). The storage of spermatozoa for 24 h in this study produced a higher result compared to a previous study by Łukaszewicz *et al*. (2020) which obtained 76.2% using EK diluent with the same storage. The viability obtained in this study was very good namely above 95% at 48 h. This value is higher than a previous study which reported that the viability of chicken spermatozoa at 48 h storage was 87.8% using fructose in egg yolk with coconut water (Rochmi and Sofyan, 2019).

The most common abnormal morphology found in this study were bent tails and bent midpieces. Abnormal morphology was reduced by the presence of glucose or fructose in ringer's acetate-yolk or ringer's lactate-yolk diluent. This is consistent with Stanishevskaya *et al*. (2021) study which stated that monosaccharides (glucose and fructose) function as an energy supply and for maintenance of the osmotic balance in sperm. Also, it is in line with another study on Tambaqui fish sperm which reported that abnormal morphology in the form of a bent tail was found to be lower in glucose-containing diluents (Oliveira *et al*., 2016). Subsequently, a previous study reported that glucose was effective in maintaining the morphology of donkey sperm (Dorado *et al*., 2019).

Abnormal sperm morphology in ringers lactate-yolk was higher compared to ringer's acetate-yolk and KA-EN 3B-yolk presumably due to the low pH in the diluent. Hofmann-Kiefer *et al*. (2012) stated that lactated Ringers solution produces a greater decrease in pH compared to acetate. The chicken sperm activities decrease at low pH (Sarkar, 2020), while acetate stimulates the process of respiration in the mitochondria of cells (Hu *et al*., 2020).

The use of ringers lactate diluent caused high abnormal morphology, this is in line with a previous study on horse sperm which explained that the addition of lactate does not improve sperm quality, but rather has a detrimental effect over time (Hernández-Avilés *et al*., 2021). Furthermore, the KA-EN 3B-yolk diluent caused the lowest abnormal morphology despite containing sodium lactate. This is presumably because it also contains 2.7% glucose which nourishes spermatozoa during storage. The abnormal morphology in the KA-EN 3B-yolk diluent at 24 and 48 h was lower compared to the result obtained from broiler chicken sperm with Lake diluent, namely 25.3-26.7% and 44.1-46.1%, respectively (Masoudi *et al*., 2019). Storage increases the abnormal morphology in spermatozoa due to an increase in reactive oxygen species (ROS). According Pintus and Ros-Santaella (2021), semen storage at 4-5^{o}C was reported to increase ROS. Thereby leading to pathological sperm morphology and lipid peroxidation (Bansal and Bilaspuri, 2010).

This study showed that the addition of sugar in ringer's acetate diluent increases the total and progressive motility, also reduces the percentage of immotile spermatozoa. This is presumably because the sugar provides an additional source of energy for spermatozoa during storage. Besides, the progressive motility of chicken sperm is highly dependent on all energy production originating from the mitochondrial compartment (Sangani *et al*., 2017). The results showed that the addition of fructose to ringer's acetate-yolk significantly improved the motility of spermatozoa. Meanwhile, Stanishevskaya *et al*. (2021) reported that fructose is a sugar that easily penetrates the cell membrane of chicken spermatozoa and creates additional energy reserves to ensure sperm motility.

The combination of ringers acetate with glucose generally produced the best spermatozoa motility parameters from all treatments. Acetate is an intermediate in many metabolic reactions and provides substrates for spermatozoa (Graham *et al*., 1982). Furthermore, chicken

sperm motility had a positive correlation with mitochondrial respiration enzyme activity and adenosine triphosphate (ATP) production (Sangani *et al*., 2017). ATP is produced through glycolysis and oxidative phosphorylation of glucose and is used as an energy source to maintain the motility of chicken spermatozoa (Setiawan *et al*., 2020).

The results obtained from this study are in line with previous studies on different species. Glucose and fructose have a strong influence on motility and movement patterns of dog spermatozoa at low-temperature storage, but glucose consumption is greater when both sugars are present in equal amounts (Ponglowhapan *et al*., 2004). Kuzlu dan Taşkin (2017) reported that glucose diluents are a good choice for a long-term storage of turkey sperm. Furthermore, the motility obtained was significantly higher than the result of another study that used Ringer's lactate diluent and had 16% motility of domestic chicken sperm at 48 h storage (Iswati *et al*., 2018). The total and progressive motility with the addition of sugar was also higher than previous studies that used tris diluent with a value of 54.04% and 15.18%, respectively, in chicken sperm (Touazi *et al*., 2018).

The type of sugar and crystalloid diluent interactions did not affect the kinematic parameters of spermatozoa except linearity and straightness. The linearity showed no significant difference with or without glucose in ringer's lactate-yolk and ringer's acetate-yolk diluents. These results are consistent with previous reports that there was no difference in the linearity of chicken sperm between the diluent without glucose and the others containing 20 mM of glucose after incubation (Setiawan *et al*., 2020). Furthermore, the straightness in ringer's acetate-yolk diluent showed no significant difference with the addition of glucose and fructose. These results are also in congruent with previous study which stated that there was no difference in the straightness of chicken spermatozoa between the diluent without glucose and others containing 20 mM of glucose after incubation (Setiawan *et al*., 2020).

The addition of glucose decreased the linearity in KA-EN 3B-yolk diluent and the straightness in ringer's lactate diluent, while the addition of fructose produced no significant differences in linearity and straightness. This is alignment with previous study on dog sperm which stated that fructose induces linear patterned movement, while glucose causes greater oscillatory movements (Rigau *et al*., 2001). The low linearity in KA-EN 3B-yolk diluent is probably due to the high glucose content in this diluent compared to ringer's lactate-yolk and ringer's acetate-yolk diluent. However, the spermatozoa kinematics in this study were higher than the previous reports, namely VSL 8.55 µm s^{-1}, VAP 17,55 µm s^{-1}, ALH 2,62 µm, and BCF 3,05 Hz using a tris diluent containing fructose at 48 h storage (Touazi *et al*., 2018). Based on all the results of our study, we found that in general the addition of glucose in ringer's acetate-yolk resulted in the best quality of spermatozoa during storage.

CONCLUSION

In conclusion, the addition of glucose or fructose in the diluent can maintain the quality of the Gaga chicken spermatozoa. The addition of glucose in Ringer's acetate yolk diluents maintains the best quality of spermatozoa during 48 h of storage in cooled storage.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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