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Seminal quality in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) with respect to weight

Araceli Cortes García, Jesús Dámaso Bustamante González and Martha Rodríguez Gutiérrez

Laboratorio de Reproducción Genética y Sanidad Acuícola, Depto. El Hombre y su Ambiente, Universidad Autónoma Metropolitana, Unidad Xochimilco (UAM-X), Calzada del Hueso 1100, Col. Villa Quietud, Coyoacán, CDMX, 04906, México.

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Abstract: In aquaculture farms, growth is an essential factor in assessing the yield of inputs, welfare of organisms and accelerating sexual maturity; the objective of the investigation was to determine seminal quality with respect to weight: one, two, three and four kilograms. Ninety-four males were anesthetized with clover essence at a concentration of 0.05 mL L^{-1} of water. The semen was extracted by light abdominal pressure in the operculum-caudal direction. The sperm concentration was determined with Neubauer chamber, viability with eosin-nigrosin, estimated in percentage and motility was evaluated by activating with water. The results indicate that increasing the weight increases the volume. In the one kilogram males the mean volume was 8.0 ± 3.9 mL, in those of two 11.1 ± 6.1 mL, in those of three 12.3 ± 8.3 mL and in those of four 29.2 ± 21.8 mL. On the other hand, the sperm concentration mL^{-1} showed an inverse tendency to the volume being the two kilograms males who showed the highest concentration $6.80 \pm 0.55 \times 10^9$ spermatozoa mL^{-1}; the highest percentage of viability 94.19 ± 1.42 % was obtained in males of two kilograms and the best motility with 86.00 ± 4.14 s in those of three kilograms. The variables presented differences ($P < 0.05$) in relation to the weight. Considering the variables of the study, it is proposed to use two kilograms organisms to ensure fertilization, which will reduce operating costs.

Keywords: Semen production; sperm concentration; sperm viability; sperm motility

Introduction

Growth and reproduction are fundamental characteristics of all living beings, which are a function of the quantity and quality of food, water and the environment. In aquaculture farms growth and reprodution are essential to optimize the performance of species for commercial interest among them, rainbow trout (*Oncorhynchus mykiss*) being one of the most cultivated in the world for its high economic value (Aral *et al*., 2007).

The production of this species in captivity is based on artificial reproduction, where the quality of gametes is fundamental to ensure a successful fertilization (Bustamante *et al*., 2016a).

The fertilizing capacity of spermatozoa can be affected by the amount of semen, sperm concentration, viability and motility among other variables (Rurangwa *et al*., 2004; Bobe and Labbé, 2010; Hajirezaee *et al*., 2010).

Consequently, the objective of the present investigation is to determine the effect of the weight in organisms of one, two, three and four kilograms with respect to the seminal quality, information that will allow to determine indicators on the variations of these variables reducing the number of reproducers, cost of maintenance and the choice of those organisms that present the best characteristics in the aquaculture centers.

Materials and Methods

Sampling

Sampling was carried out at the "El Zarco" Aquaculture Center located at the 32.5 kilometer of the México-Toluca highway (Ocoyoacac, Estado de México), 94 males of one to four kilograms were selected, which were fasted one day before sampling, for the management with anesthesia with clover essence at a concentration of 0.05 mL.L^{-1} of water (Rodríguez *et al*., 2007).

The weight (kg) of each specimen was recorded and semen was extracted by slightly applying pressure on the operculum-caudal sense. It was collected on graduated tubes for volume recording in mL and it was verified that no urine, stool, blood or water were present on the sample (Bustamante *et al*., 2016b).

Semen evaluation

The sperm concentration was determined from a

(✉) acortes@correo.xoc.uam.mx

stock solution composed of 500 mL of 4% formalin; 950 mL of 0.9% NaCl and 50 µL of semen, previously homogenized. The counting was performed in a Neubauer chamber and with the Image-Pro 5.1® program under an Olimpus Optical BX41TF® microscope (Rodríguez *et al*., 2016).

Viability was assessed based on membrane integrity, from smears of 5 µL of semen mixed with 1.5 µL of eosin-nigrosin. Red or pink stained sperm are considered alive (modified from Kuradomi *et al*., 2016).

Motility recorded in seconds under a 40X Olympus Optical BX41TF® microscope was evaluated using 1 µL of semen mixed with 5 µL of water (Rodríguez *et al*., 2016).

Statistical analysis

Study variables were processed in terms of descriptive analysis consisting of mean ± standard deviation (SD) and, as the data did not meet the normality and homoscedasticity criteria, results were analyzed with the nonparametric Kruskal-Wallis test. To establish differences, the Dunn's test was applied with a ($P < 0.05$) significance. Furthermore, a multiple correlation analysis was performed in order to define the association percentage between the weights (kg), semen production (mL) and sperm concentration variables with a ($P < 0.05$) significance level (Daniel, 2017).

Results

Weight-Semen production

The results indicate that semen production increases concomitantly with weight. The variance analysis detected significant differences ($P < 0.05$) between four kilograms males versus those weighing one, two and three kilograms (Tab. 1).

On the other hand, the analysis of variance detected significant differences ($P < 0.05$) with respect to the production of semen per kilo (Fig. 1. A).

Weight-sperm concentration

The sperm concentration mL^{-1} presented an inverse tendency to volume, with two kilograms males showing the highest concentration of 6.80 ± 0.55 x 10^9 spermatozoa mL^{-1} versus the four kilograms with the lowest 2.51 ± 0.43 x 10^9 spermatozoa mL^{-1}. Significant differences ($P < 0.05$) with respect to this variable (Tab. 1).

When evaluating the total sperm concentration, significant differences ($P < 0.05$) were determined between males of one kilogram with respect to those of two and four kilograms; however, no significant differences were found between the two and four kilograms ($P > 0.05$) (Fig. 1.B).

Tab. 1: Sperm quality in one to four kilograms males. Different upper indexes indicate significant differences ($P < 0.05$).

N	Weight (kg)	Semen production (mL)	Sperm concentration 10^9 (mL^{-1})	Sperm viability (%)	Sperm motility (s)
32	1.201 (0.199)^a	8.0 (3.9)^a	3.81 (1.21)^a	92.16 (1.25)^a	73.44 (3.04)^a
27	2.194 (0.247)^b	11.1 (6.1)^a	6.80 (1.75)^b	94.19 (1.42)^b	84.04 (2.52)^{bc}
22	3.019 (0.313)^c	12.3 (8.3)^a	4.10 (1.82)^{ac}	92.77 (1.69)^a	86.00 (4.14)^{bc}
13	4.115 (0.981)^d	29.2 (21.8)^b	2.51 (1.05)^{ad}	90.46 (1.13)^c	77.08 (2.53)^d

Viability and sperm motility

Sperm viability fluctuated between 90 and 94 % and motility between 73 and 86 s. Significant differences ($P < 0.05$) were detected in both variables with respect to some weights (Tab.1).

Relationship among variables

The association percentage among the variables weight, volume and sperm concentration was low and, in some cases, there is none (Tab. 2). This information indicates that other variables may affect semen production and sperm concentration.

Discussion

Reports of semen production and sperm concentration relative to weight in rainbow trout are scarce and results have differences in focus that volume and concentration depend on other variables such as age, genetics, stage of the reproductive period, photoperiod, water temperature among others (Bustamante *et al*., 2016a, Bobe and Labbé, 2010, Hajirezaee *et al*., 2010).

In this sense, Torres *et al*. (2014) reported 19.61 ± 0.1 mL volume for males weighing 1.5 kg. Such value is above 2 fold when compared to that obtained in this study for specimens weighing 1.201 kg.

Whereas, Bastardo *et al*. (2004) reported a mean volume of 11.97 ± 5.8 mL in males of 1,333 ± 177.9 g, lower than that reported by Torres *et al*. (2014) 19.61 ± 0.1 mL in males of 1.5 kg and higher than

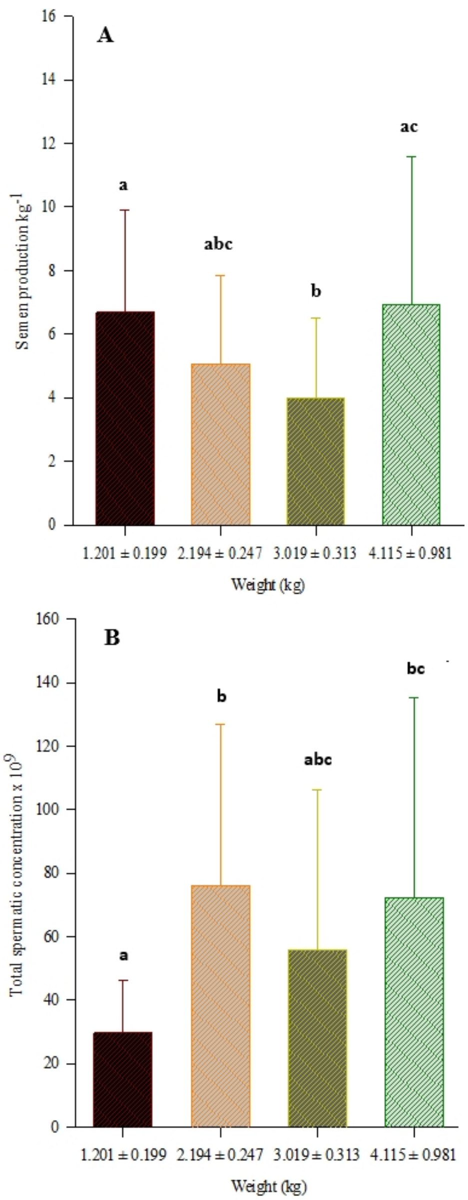

Fig. 1: A) Semen production kg^{-1} (mean ± SD); B) Total sperm concentration x 10^9 based on weight (mean ± SD). Bars displaying different upper indexes indicate significant differences (P < 0.05).

Tab. 2: Multiple correlation analysis among the variables weight, volume and sperm concentration.

Weight (kg)	Variables	r
1.201 (0.199)	Weight (kg)-Volume (mL)	0.297
	Weight (kg)-Sperm concentration	0.273
	Volume (mL)-Sperm concentration	0.179
2.194 (0.247)	Weight (kg)-Volume (mL)	0.062
	Weight (kg)-Sperm concentration	0.036
	Volume (mL)-Sperm concentration	0.107
3.019 (0.313)	Weight (kg)-Volume (mL)	0.432
	Weight (kg)-Sperm concentration	0.446
	Volume (mL)-Sperm concentration	0.386
4.115 (0.981)	Weight (kg)-Volume (mL)	0.407
	Weight (kg)-Sperm concentration	0.118
	Volume (mL)-Sperm concentration	0.044

reported in organisms of 1,201 kg of the present study. However, in Bastardo *et al*. (2004), the sperm concentration was lower than the sperm concentration reported in the present study, which was 3.81 ± 0.17 x 10^9 spermatozoa mL^{-1} (4.3 ± 1.55 x 10^6 spermatozoa mL^{-1}).

Nevertheless, Bozkurt (2006) reported an average volume of 9.3 ± 3.33 mL in organisms of 2.06 ± 0.31 kg, lower than that reported by Bustamante *et al*. (2016a) who indicate a volume of 18.01 ± 0.307 mL in males of 2.895 kg, as well as the results obtained by Torres *et al*. (2014) who obtained a volume of 19.61 ± 0.1 mL in organisms of 1.5 kg. On the other hand, Bastardo *et al*. (2004) reported a volume of 11.97 ± 5.8 mL in males of 1.333 ± 177.9 g, a volume similar to that described in the present investigation in organisms of 2.194 kg whose volume was 11.1 ± 6.1 mL.

However, although the mean volume reported by Bozkurt (2006) was below to that reported by Bastardo *et al*. (2004), the value obtained by Bustamante *et al*. (2016a) and that observed in this study displayed a higher concentration per milliliter.

On the other hand, sperm viability based on the absorption of the eosin-nigrosine dye is a function of membrane integrity Bustamante *et al*. (2016b). Methodology that has been used in several species such as: *Danio rerio* (Gerber *et al*., 2016), *Piaractus mesopotamicys* (Kuradomi *et al*., 2016), with percentages higher than 80 % of live cells as reported in the present investigation.

Another factor involved in semen quality regardless of volume and sperm concentration is the motility condition by which sperm can reach the ovum to achieve fertilization (Cosson, 2008; Dzyuba *et al*., 2013). Rurangwa *et al*. (2004); Nynca *et al*. (2012) report that the motility time is less than two minutes and highly active for less than 30 s.

In the case of rainbow trout the motility time oscillates between 30 and 60 seconds after being activated with water Cosson (2008); Nynca *et al*. (2012) less than that reported in the present investigation.

In addition, the fluctuations regarding semen production and sperm concentration values observed in this and other studies are independent of weight and they are attributed to several factors such as: culture conditions, food quality and amount, age, genetics, collecting methods, geographic conditions, reproductive season as well as environmental stimuli such as temperature and photoperiod. The latter are relevant as they depend on latitude, altitude and year season (Bustamante *et al*., 2016a; Hidahl *et al*., 2013; Bobe and Labbé, 2010; Hajirezaee *et al*., 2010; Bradshaw and Holzapfel, 2007).

Furthermore, these results revealed no correlation between weights, volume and sperm concentration per mL, thus, it is not suitable to consider either variable because low-weight specimens may produce more semen regarding those of higher weight. However, as previously mentioned the result may be similar after assessing semen production per kilogram, i.e., four kilograms reproducing specimens produced 6.9 ± 4.6 mL kg^{-1} and this value was similar to that observed for one kilogram males (6.7 ± 3.2 mL kg^{-1}), whereas sperm concentration was higher in the latter. Nevertheless, after assessing total sperm concentration the difference is evident as there is a trend indicating that volume is not a suitable marker for sperm concentration. In this regard, two kilograms males produced 11.1 ± 6.1 mL volume, whereas those weighing four kilograms produced 29.2 ± 21.8 mL, but total sperm concentration was greater in the former: 76.22 ± 50.56 y 72.38 ± 62.95 x 10^9 sperms, respectively.

Conclusion

The results demonstrates that volume and sperm concentration per mL are affected by both weight and age. Moreover, the maintenance of large-sized reproducing specimens implies more space, food and staff. Conversely, those with lower weight do not require high amounts of food, space and staff. This may decrease production costs.

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