



Effects of Ovaprim administration on reproductive parameters of Shirbot, *Barbus grypus*, Cyprinidae

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Abstract

In the present study, we have investigated the inducing effect of ovaprim on reproductive parameters of Shirbot, *Barbus grypus* including: spawning rate, working fecundity, egg weight/g.bw, egg hatching percent and larval survival rate in order to found an alternative for carp pituitary extract. For this purpose, 105 female fish were divided in 6 experimental treatments and injected intraperitoneally in two stages (first stage by 10% hormone and after 12 hours the second stage with 90% hormone) with different doses of ovaprim including: T₁: 0.25 ml/kg.bw, T₂: 0.5 ml/kg.bw, T₃: 0.75 ml/kg.bw, T₄: 1 ml/kg.bw, T₅: 1.25 ml/kg.bw, T₆: 1.5 ml/kg.bw. Also, one group was considered as control and its fish were injected by 3 mg/kg.bw pituitary extract. To obtain spermatozoa, 3 maturing male fish were injected intraperitoneally by 3 mg/kg.bw pituitary extract in one stage. According to our results, fish of T₁, T₂ and T₆ did not respond to injection and thus spawning did not occur. The highest values of spawning rate, egg weight/g.bw and working fecundity were observed in T₄ and control group. The lower values of spawning rate, egg weight/g.bw and working fecundity were obtained in T₅ compared to other experimental groups. As well as, there were no significant differences between experimental groups in terms of fertilization percent, hatching percent and larval survival rate. The results of this study suggest that a dose of 1 ml/kg.bw ovaprim and 3 mg/kg.bw pituitary extract have better and similar effects on some reproductive parameters of Shirbot such as spawning rate, egg weight/g.bw and working fecundity. Thus, use of traditional method i.e. pituitary extract could be useful yet to induce the reproduction in Shirbot.

Keywords: Ovaprim, reproductive parameters, fecundity, fertilization, pituitary extract, Shirbot, *Barbus grypus*

Introduction

The Shirbot, *Barbus grypus* is one of the important freshwater fish species in Forat and Dejles river basins. This species is nutritionally omnivorous and ecologically euryhaline and eurytherme and widely distributed in Iran, Turkey, Syria and Iraq (Nikpei, 1996; Abdoli, 2000). Shirbot is favorable among indigenous residents of south western provinces of Iran, especially Khuzestan Province (Banaee & Naderi, 2014). During last decade, the artificial reproduction of Shirbot has been conducted to restock its depleting populations in nature and also to meet market demands (Ghafleh Marammazi, 2000; Marammazi & Kahkesh 2011; Freyhof, 2014). At present, carp pituitary extract is used in the induced breeding of Shirbot, however, this method is costly and less efficient in induction of



final maturation due to the existence of some inhibitory hormones in the extract such as dopamine (reviewed by Yousefian & Mousavi, 2011). So, trends in use of synthetic hormones are increasing due to their advantages. Synthetic hormones are much less expensive and they are more stable and thus have a longer shelf life. Also, these hormones are more available in market in a purified form without any reproductive inhibitory component (reviewed by Yousefian & Mousavi, 2011). Ovaprim (sGnRHa+Domperidone) is a synthetic hormone which used widely for spawning induction of cyprinidae and other commercial fish species. Seifi, Imanpoor, Jafari and Makhdomi (2011) demonstrated that artificial induction of maturation by ovaprim enhances milt quality parameters in common carp, *Cyprinus carpio*. More, Bhandare, Shinde, Pathan, & Sonawane (2010) have investigated the ovaprim-induced maturation of three Indian carps, *Catla*, *Rohu* and *Mrigal*. These authors showed that a single intra-muscular injection of ovaprim (0.5-0.2 ml/kg.bw) can induces final maturation. Also, more percentage of fertilization (75-90%) was found with ovaprim compared to pituitary extract (65- 80%). In the Asp, *Aspius aspius*, the highest percentage of ovulation (100%) and embryo-survival to the eyed-egg-stage (81.3%) was recorded after the application of a combination of ovopel and ovaprim in compared to other groups. As well as, fish from the control group did not ovulate. The latency time was shorter in the groups where ovopel and ovopel with ovaprim was applied (40 h) than in ovaprim group (42–44 h) (Targońska, Kucharczyk, Zarski, Mamcarz, & Falahatkar, 2011). Naeem, Salam, and AJafar (2005) suggested that a single dose of ovaprim (0.7 mg/ kg.bw) successfully induces reproduction in *Catla catla*. In *Barbus sharpeyi*, intramuscular injection of LHRHa+CPE (Carp Pituitary) had more efficiency on reproductive parameters than ovaprim (Kahkesh, Yooneszadeh Feshalami, Amiri, & Nickpey, 2010). Gharaei, Abdolali, and Mostafa (2011) concluded that ovaprim is the most suitable for inducing spawning in Snow trout, *Schizothorax zarudnyi* when they observed more working fecundity and percentage of eyed egg to larvae in ovaprim treated group. Mabudi, Savari, and Javadzadeh (2013) demonstrated that 1.25 ml/kg.bw ovaprim improves spawning and fertilization rate in *Barbus xanthopterus*. To our knowledge, there are no studies regarding the inducing impacts of ovaprim on reproductive parameters of Shirbot, *Barbus grypus*. Thus,

In the present study, we examine the effects of various doses of ovaprim on reproductive properties of Shirbot. Such study can help to aquaculture enhancing of Shirbot.

Material and Methods

Experimental design:

The experiment was conducted at developmental center for endemic fishes, Susangerd, Ahvaz, Iran. Broodstocks of Shirbot (n=105 females, (total weight = 1600 ± 100 g); n=3 males (total weight = 4260 ± 450 g)) were captured from tributaries of Karkheh and Karun Rivers. The Broodstocks were transferred to hatchery by 1000 l tank supplied with oxygen. Then, females and males were separated and stocked in different ponds to prevent natural spawning.



Female fish were identified through morphological characteristics such as pink to red and protruding genital papilla and distended abdomen. In matured males, sperm was released from genital papilla with slight pressure on the belly. In the present study, the cultured males with same size and age were considered for experiment. After 2 days acclimation to hatchery condition, female fish were injected intraperitoneally in two stages (first stage by 10% hormone and after 12 hours the second stage with 90% hormone) with various doses of ovaprim (20 µg GnRH+ 10 mg Domperidone, Asia-Company, Iran) depending on experimental treatments. In this regards 6 experimental treatments including: T₁: 0.25 ml/kg.bw, T₂: 0.5 ml/kg.bw, T₃: 0.75 ml/kg.bw, T₄: 1 ml/kg.bw, T₅: 1.25 ml/kg.bw, T₆: 1.5 ml/kg.bw were designed. Also, one group was considered as control and its fish were injected intraperitoneally by 3/kg.bw pituitary extract. Also, male fish were injected intraperitoneally by 3/kg.bw pituitary extract in one stage when females received their last injection. Before hormone injection, the fish were anaesthetized by ethylene glycol monophenyl ether (30 ml/100 l water) (Gyes weii, 1992) and then hormones injected by 3 ml syringe. After final injection, fish were checked every 6 h interval up to ovulation. During the course of the experiment, the water condition (temperature, dissolved oxygen and pH) of each pond was checked and maintained in normal range. After 12 h, the ovulation occurred and egg collection carried out by hand-stripping. Before egg collection, fish were anaesthetized by ethylene glycol monophenyl ether (200 ppm) (Sarker & Satoh, 2007). The eggs were fertilized according to Billard, Cosson, Perchec, and Linhart (1995) by pooled milt samples of three males and then incubated separately in Veis incubators (250 g egg per Veis incubator) until hatching i.e. 3-4 days after fertilization.

Reproductive parameters:

The reproductive indices were spawning rate (%), egg weight/g.bw, working fecundity, fertilization percent (%), hatching percent (%) and larvae survival percent (%). These indices were measured as follow formula:

Spawning rate = The number of ovulated fish/total number of injected fish) × 100

Fertilization rate = Number of fertilized egg/ total eggs × 100.

Fertilization rate was determined under a dissecting loop 8h after fertilization, when were at the stage of gastrulation (Brommage & Cumalantunga, 1998).

Hatching percent = Number of viable embryos/total number of eggs) × 100 (23) (Hanjavanit, Kitancharoen, & Rakmanee, 2008)

Working fecundity = The number of collected eggs/Kg.bw)

Egg weight/g.bw= The number of collected eggs (g)/total body weight (g)



Statistical Analysis:

SPSS software (Ver.16) was used for data analysis. Data normality was investigated by Kolmogorov–Smirnov test. Differences between means were analyzed by one way analysis of variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significant of $P < 0.05$. Data measured in percentage scale were converted by angular transformation ($\arcsin \sqrt{p}$) prior to analysis. All results are presented as means \pm standard error of the mean.

Results

According to our results, fish of T_1 , T_2 and T_6 did not respond to injection and thus spawning did not occur. There were significant differences between experimental groups in terms of spawning rate (Fig 1), working fecundity (Fig 2) and egg weight/g.bw (Fig 3) ($P < 0.05$). In this regard, the highest values of spawning rate were observed in T_4 and control group (Fig 1, $P < 0.05$). Also, there were no significant differences between T_4 and control group (Fig 1, $P > 0.05$). Fish of T_5 showed the lowest spawning rate among all experimental groups (Fig 1, $P < 0.05$). The highest values of egg weight/g.bw obtained in fish administrated with ovaprim (1 ml/kg.bw: T_4) and with pituitary extract (3 mg/kg.bw: control) compared to other experimental groups (Fig 3, $P < 0.05$). The lower values of egg weight/g.bw yielded in T_5 compared to other experimental groups (Fig 3, $P < 0.05$). Similar to spawning rate and egg weight/g.bw, more working fecundity was found in T_4 and control group (Fig 2, $P < 0.05$). Also, the lowest working fecundity was observed in T_5 (Fig 2, $P < 0.05$). As well as, there were no significant differences between experimental groups in terms of fertilization percent (Fig 4), hatching percent (Fig 5) and larval survival rate (Fig 6) ($P > 0.05$).

Discussion

In the present study, a combination of GnRH and Domperidone (as anti-dopamine) was applied for first time for inducing the spawning in Shirbot. Since the inhibitory action of dopamine on LH (luteinizing hormone) secretion is very strong in cyprinids, the use of dopamine antagonists combined with GnRH agonists is necessary to induce the ovulation in a sufficient percentage of fish brooders (De Leeuw, Reasink, Rooyackers, & Goos, 1985; Mikolajczyk, Chyb, Sokolowska, Mikolajczyk, Enright, Epler, Filipiak, & Breton, 2003). The use of various dopamine antagonists to induce ovulation in cyprinids fish is a well-known method in aquaculture (Peter, Chang, Nahorniak, Omeljaniuk, Sokolowska, Shih, & Billard, 1986; Peter & Yu, 1997; Barth, Justice, & Ngai, 1997; Brzuska, 2000). Spawning rates, working fecundity and egg weight/g.bw in the present study were between 40-80%, 6200-13300, 130-240. The highest values of spawning rate, working fecundity and egg weight/g.bw were observed when fish administrated with 1 ml/kg.bw ovaprim and 3 mg/kg.bw pituitary extract. According to previous studies, the responses of cyprinid fishes to ovaprim are different. For example, Nandeesh, Rao, Jayanna, Parker, Varghese, Keshavanath, and Shetty (1990) obtained the best results in inducing reproduction of Indian carps at dose of 0.3-0.4 ml kg ovaprim. However



for the Snow trout a much higher dose of 1.5 ml/kg.bw made the best results (Gharaei et al., 2011). These results along with our results indicate that reproductive responses to ovaprim are dose-dependent and are different depending on kind of species. In our study, the spawning did not occur in fish of administrated with 0.25, 0.5 and 1.5 ml/kg.bw ovaprim. In fact, ovaprim both in low and high doses had no inducing effect on reproduction of Shirbot. Some studies demonstrated that induction of fish spawning by using hormonee therapy had not been successful when the hormones had been used in much low and high doses (Rottmann, Shireman, & Chapman, 1991). In low doses, a reproductive hormone cannot support all maturation events such as: germinal vesicle migration and breakdown and subsequently ovulation (Rottmann et al., 1991). Also, some authors have indicated that the reproduction is impaired due to the physiological negative feedbacks on pituitary gland when the high doses of reproductive hormones are used; as it was found in fish of T₆ (1.5 ml/kg.bw ovaprim) in our study. Therefore, a high dose of ovaprim in our study might affect the pituitary gland as a negative signal and suppress the secretion of gonadotropin. In the present study, since the highest values of spawning rate, working fecundity and egg weight/g.bw were obtained in fish administrated with 1 ml/kg.bw ovaprim, thus this dose is suggested for artificial induction of Shirbot. However, non-significant differences between T₄ and control group in terms of spawning rate, working fecundity and egg weight/g.bw suggest that the commonplace method i.e. use of pituitary extract could be an useful method yet for induction of spawning in Shirbot. In addition, this result shows that the inhibitory action of dopamine in Shirbot is probably weak compared to other cyprinid fishes. In our study, there were no significant differences between experimental groups in terms of fertilization percent, hatching percent and larval survival rate. This case suggests that the viability of eggs after ovulation is independent from dose of applied hormone. In conclusion, the results of the present study showed that ovaprim at dose of 1 ml/kg.bw improves more the reproductive parameters of Shirbot. As wells, the inhibitory action of dopamine is probably low in this species since the results in fish administrated with 1 ml/kg.bw ovaprim were similar to those administrated with 3 mg/kg.bw pituitary extract.

References

- Abdoli, A. (2000). The inlandwater fishes of Iran. Iranian Museum of Nature and Wildlife, Tehran. 378P (in Persian).
- Banaee, M., & Naderi, M. (2014). The reproductive biology of shirbot (*Barbus grypus* Heckel, 1843) in the Maroon River, Iran. *International Journal of Aquatic Biology*, 2(1), 43-52.
- Barth, A. L., Justice, N. J., & Ngai, J. (1996). Asynchronous onset of odorant receptor expression in the developing zebrafish olfactory system. *Neuron*, 16(1), 23-34. [http://dx.doi.org/10.1016/S0896-6273\(00\)80020-3](http://dx.doi.org/10.1016/S0896-6273(00)80020-3)
- Billard, R., Cosson, J., Perchec, G., & Linhart, O. (1995). Biology of sperm and artificial reproduction in carp. *Aquaculture*, 129(1-4), 95-112. [http://dx.doi.org/10.1016/0044-8486\(94\)00231-C](http://dx.doi.org/10.1016/0044-8486(94)00231-C)
- Kahkesh, F. B., Feshalami, M. Y., Amiri, F., & Nickpey, M. (2010). Effect of ovaprim, ovatide, HCG, LHRH-A2, LHRHA2+ CPE and carp pituitary in Benni (*Barbus sharpeyi*) artificial breeding. *Global Veterinaria*, 5(4), 209-214.



- Bromage, N., & Cumarantunga, R. (1988). Egg production in the rainbow trout. In *Recent advances in aquaculture* (pp. 63-138). Springer Netherlands. http://dx.doi.org/10.1007/978-94-011-9743-4_2
- Brzuska, E. (1979). The in vivo method of estimating the stages of oocytes maturation in carp *Cyprinus carpio* L. *Acta Hydrobiol*, 21, 423-433.
- De Leeuw, R., Resink, J. W., Rooyackers, E. J. M., & Goos, H. T. (1985). Pimozide modulates the luteinizing hormone-releasing hormone effect on gonadotrophin release in the African catfish, *Clarias lazera*. *General and comparative endocrinology*, 58(1), 120-127. [http://dx.doi.org/10.1016/0016-6480\(85\)90144-3](http://dx.doi.org/10.1016/0016-6480(85)90144-3)
- Freyhof, J. 2014. *Barbus grypus*. The IUCN Red List of Threatened Species 2014: e.T19171241A19223133. <http://dx.doi.org/10.2305/IUCN.UK.20141.RLTS.T19171241A19223133.en>. Downloaded on 22 February 2016.
- Ghafleh M.J. (2000). Feeding and Spawning Characters of *Barbus grypus* Heckel, 1843 in Khouzesan Water Bodies. *Iranian Journal of Fisheries Sciences*, 9, 67-80.
- Gharaei, A., Rahdari, A., & Ghaffari, M. (2011). Induced spawning of *Schizothorax zarudnyi* (Cyprinidae) by using synthetic hormones (Ovaprim and HCG). *World Journal of Fish and Marine Sciences*, 3(6), 518-522.
- Hanjavanit, C., Kitancharoen, N., & Rakmanee, C. (2008). Experimental infection of aquatic fungi on eggs of African catfish (*Clarias gariepinus* Burch). *KKU Science*, 36, 36-43.
- More, P. R., Bhandare, R. Y., Shinde, S. E., Pathan, T. S., & Sonawane, D. L. (2010). Comparative study of synthetic hormones ovaprim and carp pituitary extract used in Induced breeding of Indian major carps. *Libyan Agric. Res. Cen. J. Intl*, 1(5), 288-295.
- Marammazi, J. G., & Kahkesh, F. (2011). Effects of dietary protein and energy levels on growth performance. *Iranian Journal of Fisheries Sciences*, 10(3), 461-474.
- Mikolajczyk, T., Chyb, J., Sokolowska-Mikolajczyk, M., Enright, W. J., Epler, P., Filipiak, M., & Breton, B. (2003). Attempts to induce an LH surge and ovulation in common carp (*Cyprinus carpio* L.) by differential application of a potent GnRH analogue, azagly-nafarelin, under laboratory, commercial hatchery, and natural conditions. *Aquaculture*, 223(1), 141-157. [http://dx.doi.org/10.1016/S0044-8486\(03\)00158-3](http://dx.doi.org/10.1016/S0044-8486(03)00158-3)
- Nandeesh, M. C., Rao, K. G., Jayanna, R. N., Parker, N. C., Varghese, T. J., Keshavanath, P., & Shetty, H. P. (1990). Induced spawning of Indian major carps through single application of Ovaprim-C. In *the second asian fisheries forum*, (Eds: R. Hirano and M. Hanyu). *Asian Fisheries Society, Manila, Philippines* (pp. 581-585).
- Naeem, M., Salam, A., & Jafar, A. (2005). Induced spawning of major carp *Catla catla* by a single intramuscular injection of ovaprim-c and fecundity at fish hatchery Islamabad. *Pakistan. Journal of Biological Sciences*, 5(6), 776-780. <http://dx.doi.org/10.3923/jbs.2005.776.780>
- Nikpei, M (1996). Research project report: biological study of *Barbus grypus* and *Barbus sharpeii*. Iranian Fisheries Research Institute. 1: 52-64.
- Peter, R. E., Chang, J. P., Nahorniak, C. S., Omeljaniuk, R. J., Sokolowska, M., Shih, S. H., & Billard, R. (1986). Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. *Recent progress in hormone research*, 42, 513. <http://dx.doi.org/10.1016/B978-0-12-571142-5.50016-1>
- Peter, R. E., & Yu, K. L. (1997). Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Reviews in Fish Biology and Fisheries*, 7(2), 173-197. <http://dx.doi.org/10.1023/A:1018431610220>
- Rottmann, R. W., Shireman, J. V., & Chapman, F. A. (1991). *Hormonal control of reproduction in fish for induced spawning*. Southern Regional Aquaculture Center.
- Mabudi, H., Savari, A., & Javadzadeh, N. (2013). The integrated effect of LHRHA2 and pituitary extract on maturation of *Barbus xanthopterus*. *International Journal of Marine Science Engineering*, 3, 153-158.



Sarker, M. A., & Satoh, S. (2007). Influence of Dietary Phosphorus and Zinc Levels on Whole Body Mineral, Liver Mineral, and Liver Vitamin-C Contents of Fingerling Rainbow Trout, *Oncorhynchus mykiss*. *Journal of Agriculture & Rural Development*, 5(1), 135-142. <http://dx.doi.org/10.3329/jard.v5i1.1470>

Seifi, T., Imanpoor, M.R., Jafari V., & Makhdomi, C. (2011). The injection effects of ovaprim, HCG and pituitary extract hormones on Sperm quality in cultured common carp (*Cyprinus carpio* Linnaeus, 1758). *Journal of Fisheries, Iranian Journal of Natural Resources*, 64, 55-63 (in persian).

Targońska, K., Kucharczyk, D., Żarski, D., Mamcarz, A., & Falahatkar, B. (2011). Optimization of artificial reproduction of Asp, *Aspius aspius* (L.) under controlled conditions. *Polish Journal of Natural Sciences*, 26(2).

Mehdi, Y., & Ehsan, S. (2011). A review of the control of reproduction and hormonal manipulations in finfish species. *African Journal of Agricultural Research*, 6(7), 1643-1650. <http://dx.doi.org/10.5897/AJAR10.1080>

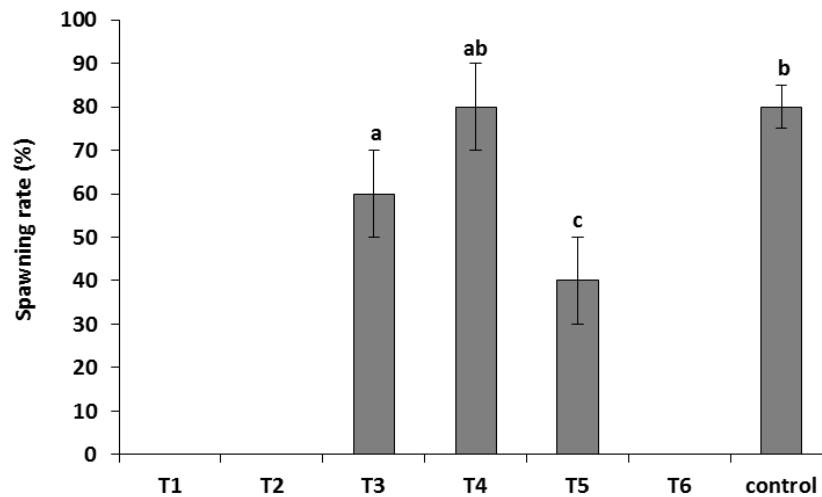


Figure 1. Comparison of spawning rate (%) of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).

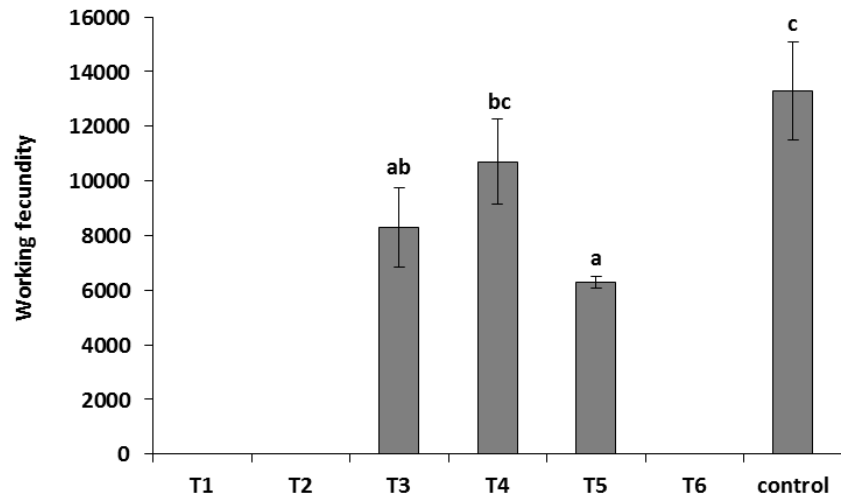


Figure 2. Comparison of working fecundity of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P < 0.05$).

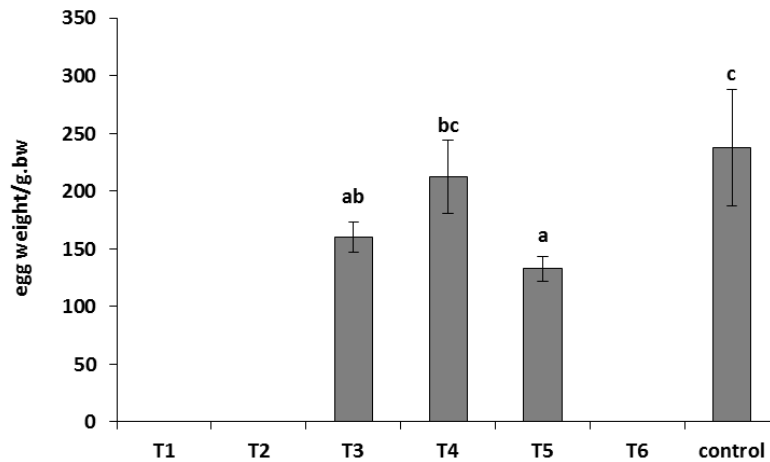


Figure 3. Comparison of egg weight/g.bw of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P < 0.05$).

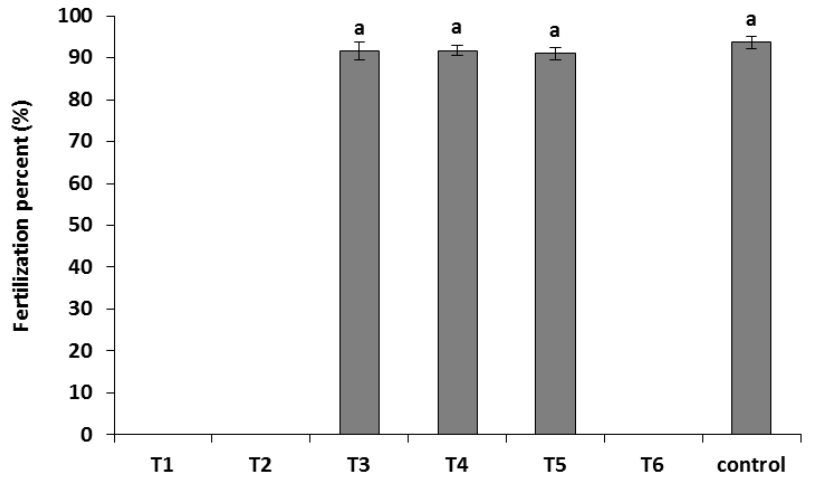


Figure 4. Comparison of fertilization percent of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).

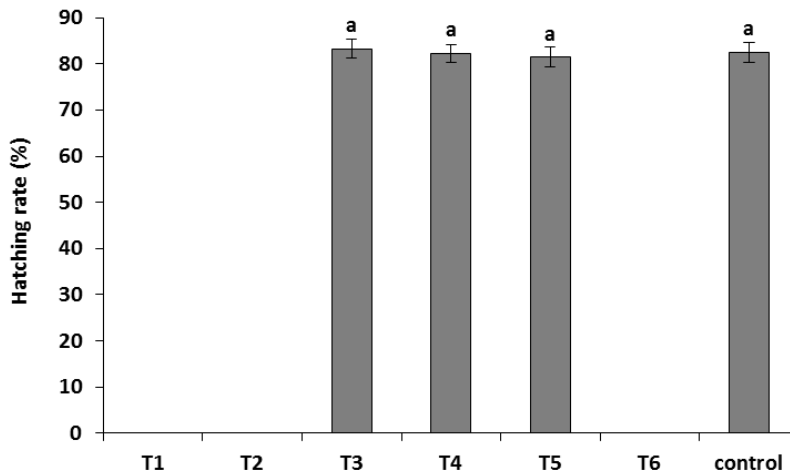


Figure 5. Comparison of hatching percent of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).

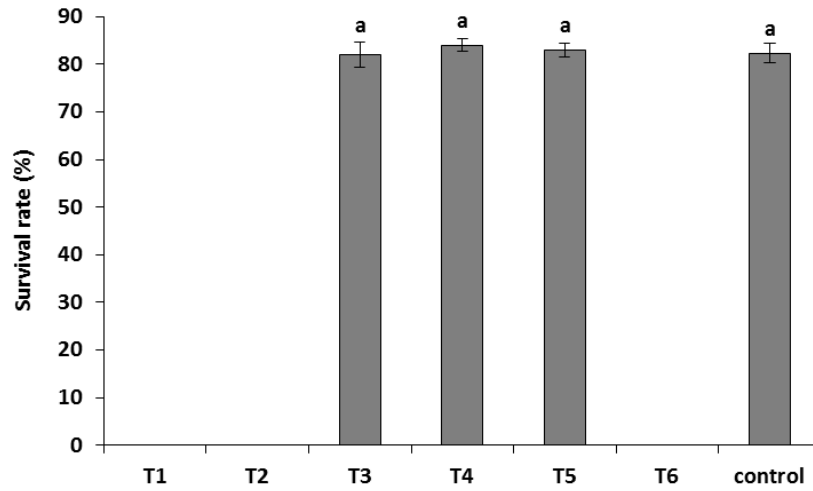


Figure 6. Comparison of larval survival rate (%) of Shirbot between experimental groups. T₁: fish administrated with 0.25 ml/kg.bw ovaprim, T₂: fish administrated with 0.5 ml/kg.bw, T₃: fish administrated with 0.75 ml/kg.bw, T₄: fish administrated with 1 ml/kg.bw, T₅: fish administrated with 1.25 ml/kg.bw, T₆: fish administrated with 1.5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).