Global Veterinaria 5 (4): 209-214, 2010
ISSN 1992-6197

Effect of Ovaprim, Ovatide, HCG, LHRH-A2, LHRHA2+CPE and Carp Pituitary in Benni (*Barbus sharpeyi*) Artificial Breeding

Froud Bosak Kahkesh, Mohammad Yooneszadeh Feshalami, Farokh Amiri and Mansur Nickpey

South Iran Aquculture Research Center, Ahvaz, 61645/866, Khuzestan, Iran

Abstract: The *Barbus sharpeyi* is a species of the genus Barbus of cyprinidae with local name"Benni" distributed in shadegan and horolazim GnRHA2-effectiveness of Ovaprim, Ovatide, GnRHA2, LHRH-A2, LHRHA2+CPE and Carp Pituitary on spawning success, Latency period, working fecundity, fertilization success and hatching rate. 56 fish were divided into 7 treatments and injected intramuscullary as follows. 4mg kg^{-1} b.w.of CPE as positive control, Propylene glycol as negative control, 0.5 mg kg^{-1}b.w. of Ovaprim, 0.5 mg kg^{-1}b.w. of Ovatide, 1000 Iu kg^{-1}b.w. of HCG, 10µg kg^{-1}b.w. of LHRH-A2, 10µg+2 mg kg^{-1} b.w. of LRHa+CPE in double injection 10 h apart. Results showed that LRHa+CPE combination yielded 87.5% spawning success in comparison with HCG, Ovaprim, Ovatide, LRHa and CPE. None of fish were ovulated in the groups of negative contol, HCG and LRHa, while 3/8 fish were ovulated in the group of Ovaprim and Ovatide(37.5%). 6/8 fish were ovulated in the group of CPE(75%). Therefor, LHRHa+CPE combination can be effected comparison CPE or alone other hormones.

Key words: Barbus sharpeyi · Spawning · LHRHa · HCG · Ovaprim · Ovatide

INTRODUCTION

The *Barbus sharpeyi* (Al. Hassan L.A.J 1983) is a species of the genus Barbus of cyprinidae and is widely distributed in the Syria, Iraq, Turkey, Iran, Nile, Victoria and Naser river [1]. In Iran, this species with local name "Benni" is distributed at shadegan and horolazim wetlands in the southwest of Iran. It has a synchronous single behaviour spawning on aquatic weeds in kharkhe river [2]. This is a very valuable commercial fish in the southwest of Iran and is greatly demanded due to its good taste and culinary customs of the local people. To restock this valuable species in the wetlands, the Iranian Fisheries Organization (shilat) produced and release up to 3.5 million fry (average weight 1g) in the horolazim wetland annually [3].

Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in the fish farming industry for two main reasons; to solve the problem of spawning asynchrony which necessitates frequent broodstock handling [4,5] and for accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed [6] Use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs [7].

Originally, culturists utilized carp pituitary (CP) and this is still widely used particularly for the major Indian carps, Chinese carps and the common carp *Cyprinus carpio* [6, 8]. Human chorionic gonadotropin (HCG) has been used to induce final maturation of oocytes and also as a tool for utilization in commercial in aquaculture [7-11].

The superactive luteinizing hormone-releasing hormone analogue des-Gly10[D-Ala6]LHRHEthylamide (LHRHa) has been successfully used to induce final maturation and synchronize ovulation of many commercially cultured fish [9, 12]. The use of different forms of gonadotropin releasing hormone agonist (GnRHa), which stimulate secretion of endogenous gonadotropin(GTH) [13, 14] Ovaprim and Ovatide are a kind of analogue of salmon gonadotropin realasing hormone (sGnRHa) with a dopamine blocker [15]. The use of sGnRHa resulted in successful stimulation of ovulation in some of cyprinids [16-19] and catfishes [20]. The objective of induced ovulation is to produce, on demand, a large supply of high quality eggs. Egg quality is assessed by characteristics such as egg fertility and hatching [21]. Hormonal induction of final oocyte maturation and ovulation, however, can result in reduced egg quality [22].

Corresponding Author: Mohammad Yooneszadeh Feshalami, South Iran Aquculture Research Center, Ahvaz, 61645/866, Khuzestan, Iran, E-mail: m_yooneszadeh@yahoo.com.

In the present study we investigated the effects of hormone of HCG, LHRHa, Ovaprim, Ovatide, LHRHa+HCG and CP on spawning success, spawning success, Latency period, working fecundity, fertilization success, hatching rate and survival larval rate, in order to develop simple and cost effective method for accelerating and synchronizing ovulation in *B. sharpeyi.*

MATERIALS AND METHODS

Fish Stocks and Maintance: The experiment were conducted at south Iran aquculture Research Center, Ahvaz, Khozestan. Iran. Benni were captured from the Horolazim Wetland and maintained in earth pond in January 2008 (water temperature 15-17°C). 56 female fish weighing 800-2000 g body weight (b.w.) were used. Females were selected for injections in May based on external characteristics reddish swollen vent and a soft rounded abdomen. Prior to injection, fish were individually weighted and marked by colour cloths on the tail fin and wee randomly divided into treatment groups.

Hormones: Ovaprim (Syndel International Inc., Canada) is a liquid prepation containing salmon GnRH analogue (D-Arg^{6}, Pro^{9} Net-sGnRH) and domperidone, a dopamine antagonist. The manufacturers recommened dose is 0.5 ml/ kg^{-1}b.w of spawner body weight. The Ovatide (sGnRH+Dopamin) was supplied by Institute of Fisheries Education, Mumbai, India.

Luteinizin Hormone-Releasing hormone analogue (Des-Gly10, [D-Ala 6] LH-RH Ethylamide) or LHRHa is a peptide that is similar in structure to native luteinizing hormone hormones (LHRH). The LRHa available on the market is a white powder and is combined with mannite as a filler (made in China).

Human chronic gonadotropin (HCG) is a polypeptide hormone with molecular weight 36000. At present, the ready-made material available on the market in china is "veterinary gonadotropin".

Experiments: Groups of 8 fish were injected I.M. with different preparation: CPE as a control group (3 mg kg^{-1}b.w), HCG alone 1000 Iu kg^{-1}b.w in double injections, Ovaprim and Ovatide alone 0.5ml/ kg^{-1} in a single injection, LHRHa alone 10 µg kg^{-1} in double injection, LHRHa combined with CPE (10 µg kg^{-1}+1.5 mg kg^{-1} b.w) in double injection. Double injection were done in 10-90% ratio,10 h apart.

After injection, the fish were placed in an indoor fiberglass tank with running water, temperature 23-24°C. The fish were checked for ovulation after first injection every 13h interval up to ovulation. Although, they can spontaneously spawn in the tank after hormonal induction, but because of the large number of broodfish in hatchery and stickiness of eggs, it is better to enhance gamete quality and quantity. So when ovulation was observed, the eggs were stripped manually and fertilized with milt from at least two males and 250-300 g of fertilized eggs from each female was incubated in vase (7 liter) incubators up to hatching.

Spawning rate (the number of ovulated fish/total number of injection fish) and embryo viability percent (number of viable embryos/total number of eggs×100) were determined [23]. The latency period (the time between the first injection and fish ovulation and working fecundity (the number of stripped eggs/kg b.w.) was calculated [16, 24], respectively.

Fertilization rate was determined under a dissecting loop 8h after fertilization, when were at the stage of gastrulation.

Statistical Analysis: Spawning rate was analyzed by the Chi-square test [25]. Differences in latency period, working fecundity, fertilization rate and hatching rate were analyzed by one way analysis of variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significant of $P<0.05$. Results are presented as means ± standard error of the mean (S.E.M).

RESULTS

Non of 8 fish ovulated in the HCG and LHRHa groups after injection (Table 1). In the control group, six out of 8 ovulated (75%). Three out of 8 ovulated (37.5%) in the Ovaprim and Ovatide groups. The lowest spawning (0%) in the HCG and LHRHa groups were observed. Combination of LHRHa+CPE was most effective for induction and the highest spawning rate (87.5%).

The latency periods were in the range of 24-26.6 h after the first injection. The mean latency period was 24.42±0.2 h in LHRHa+CPE group which was lower than all other groups ($P<0.05$). The longest period (25.38±0.2 h) was observed in the control group(CPE). The mean latency period was 25.23±0.33 h in ovaprim (T6) and similar result achieved by the ovatide treatment (Table 1). The mean working fecundity h in ovulated fish is shown in Table 1. The mean working fecundity h in treatments were 20625-73333 and LHRHa+CPE was the highest working fecundity among groups ($P<0.05$).

Table 1: The effect of different hormone treatment on spawning success (%), latency period (h), fertilization rate ovulation index OI(%) and (%) of Benni, *Barbus sharpie*

		Dosage						
Treatment ID	Treatment	1 st	2nd	Spawning success(%)	Latency period	Working fecundity	Fertilization success (%)	Hatching rate (%)
Positive control	CPE	0.3mg	2.7mg	75[c]	25.38±0.2[b]	33687.49±2960[a]	82.83±4.36[a]	66.16±3.49[a]
Negative control	Propylene glycol	-	-	-	-	-	-	-
T1	HCG (1000)	100Iu	900Iu	0[a]	-	-	-	-
T2	LRHa(10)	1µ	9µ	0[a]	-	-	-	-
T3	LRHa+CPE(10+2)	1+0.2	9+1.8	87.5[c]	24.42±0.2[a]	56626.18±5036[b]	94.57±0.99[b]	78.42±1.65[b]
T4	Ovatide	0.5ml/kg		37.5[b]	25.2±0.1[b]	34774.28±1163[a]	84.33±2.33[ab]	76±1.15[b]
T5	Ovaprim	0.5ml/kg		37.5[b]	25.2±0.1[b]	33783.28±1363[a]	85.26±2.54[ab]	77±1.36[b]

Mean(± S.E.M.) value with a different letter are significantly different ($p<0.05$)

Fertilization rate in treated fish was in the range of 84.33-94.57% (Table 1) and CPE was lowest rate 82.83±4.36 among groups ($P<0.05$). There was significant difference in fertilization success among groups ($P<0.05$). The Hatching rate% was in the range 66-78 % and showed significantly difference among groups (Table 1). The cotrol group showed the lowest hatching rate ($P<0.5$).

DISCUSSION

The necessity of using inducing agents such as CPE, HCG, LHRHa and sGnRH (ovaprim) for induction of spawning has been demonstrated in cyprinid fish such as common and Chinese carps [10, 26-28] as well as Indian major carps [27-30]. Benni reproduction in captivity requires hormonal stimulation. To data, there have been no reports of obtaining oocytes from female with it. The spawning success was different among groups (Table 1), with 87.5% spawning success in LHRHa+CPE treatment, a value higher than positive control and other treatments. As reported Ovaprim and Ovatide is a known spawning inducing agent in Indian major carps, catfish and other carp species [10, 31-33] and the highest ovulation (100%) in Nase, Chondrostoma nasus [34]. Non of fish ovulated in the negative control, HCG and LHRHa treatments. As a result, it is proposed to combine LHRH+CPE was best treatment for successful spawning induction. HCG alone was ineffective except when combined with carp pituitaryhomogenate in hypophysectomized goldfish [35].

HCG alone or in combination with fish pituitary induced spawning in silver carp and rohu [36, 37]. Chonder [38] has described the technique of repeated breeding in Indian and Chinese major carps during the same spawning season by administering HCG injections [38].

This success immediately gained international attention and LHRH-A has been successfully used for maturation and spawning of various fish including coho salmon [39], Atlantic salmon [4], seabass and rabbit fish [40] and milkfish [41]. Successful spawning through a single dose of ovaprim has also been reported is several species of fish in India [31, 32]. Overall, the results of this study showed that in B. sharpyei ovulation could not be successfully induced with hCG and LHRHa alone. LHRHa with CPE induced ovulation (85%) in female compared to female that received LHRHa alone. To data showed that ovatide and ovaprim were able to induced ovulation in 37.5% of the female *B. sharpyei*.

Despite research in our laboratory suggesting that LHRHa about 100% of chines carp ovulate [28] in response to a second dose of 10 µg/kg LHRHa, in this study B. sharpyei failed to respond to a similar treatment. Why the response to hormones was different in B. sharpie to that typically found in Chinese carp is not known. This search was similar to results that carried out on *B. xanthopetrus* [42]. The latency period was observed 24-26 h in treatments that responded to hormones. The latency period were greater than reported for catfish [43, 44], common carp [45-47], chines carp [48] and lower than reported for spotted murrel [44] Kutum [49], Nase [34]. Assessment of effectiveness of hormonal treatments can be done by examining spawning success, work fecundity, fertilization success and hatching success after hormonal treatments. According to our results the work fecundity in spawned fish was approximately in the range of 20-73 thousands. LHRHa+CPE was the highest working fecundity among groups, while CPE treatment was the lowest working fecundity. The work fecundity was lower than reported for common carp [43].

As reported Ovaprim (sGnRHA) is a known spawning inducing agent in Indian majorcarps and other carp species [10, 31].

"Linpe" method (sGnRHA and dopamine antagonist) has been used for induced ovulation in case of a number of cultured fish [10].

Fertilization success showed significant differences between CPE with LHRHa+CPE treatment ssuggesting that LHRHa+CPE was greatest fertilization success (94.57%). The percentage of fertilization and hatching rate remained consistently higher than ovaprim, ovatide and LHRHa+CPE treatments as compared to CPE in the trials. One of the reasons for this difference is the poor quality of pituitary glands used in various farms [32].

The type of hormones, administration protocols and gamete acquisition procedures may vary depending on the reproductive biology of each cultured species and a thorough understanding of the endocrine controle of gametogenesis,final maturation and spawning is essential for the appropriate management of the species [7]. In different dosage LHRHa in Gattan *Barbus xanthopetrus* was not show change in spawning success. That showed LHRHa were not effect only spawning success [42].

Recently, HCG preparation has been approved for commercial utilization in commercial aquculture [7].

In conclusion, this study demonstrated that a combination of LHRHa+CPE is an effective and reliable method for induction of ovulation in benni and can be very useful for hatchery and broodfish management, spawning and restocking programs. The advantage over the of CPE include its greater availability and lower cost.

ACKNOWLEDGEMENTS

We wish to thank the workers of the South Iran Aquculture Research Center, Ahvaz, Iran.

REFERENCES

1. Hashem, M.T. and A. Agamy, 1977. Effect offishing and maturation on Barbus bynni population of Nozha Hydrodrom. Bull. Inst. Ocean and Fish, 7:137.
2. Nickpey, N., G. Eskandari and S. Dehghan, 1997. The survey of Barbus grypus and *B. sharpryi* biology. Iranian Fisheries Research Organization, Tehran, pp: 1-64.
3. Shilat, Iranian Fisheries Organization (www.shilat.com).
4. Crim, L.W. and B.D. Glebe, 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture, 43: 47-56.
5. Lin, H.R. and R.E. Peter, 1996. Hormones and spawning in fish. Asian Fisheries Sci., 9: 21-23.
6. Lam, T.J., 1983. Environmental influences on gonadal activity in fish. In: Fish Physiology ? B (eds. W.S. Hoar, D.J. Randall and E.M. Donaldson), pp: 65-116. Academic Press, London.
7. Mylonas, C.C., A. Fostier and S. Zanuy, 2009. Broodstock management and hormonal manipulation of fish reproduction. Aquculture, 197: 99-136.
8. Park, I.S., H.B. Kim, H.J. Choi, Y.D. Lee and H.W. Kang, 1994. Artificial induction of spawning by human chorionic gonadotropin (HCG) or carp pituitary extract (CPE) in olive flounder, Paralichthys olivaceus. J. Aquaculture, 7: 89-96.
9. Donaldson, E.M. and G.A. Hunter. 1983. Induced final maturation, ovulation and spermation in cultured fish. In: W.S. Hoar, D.J. Rondall and E.M. Donalson, (Eds.), Fish Physiology. Vol. IX, Part B:Reproduction. Academic Press, Orlando, Florida, pp: 351-403.
10. Peter, R.E., H.R. Lin and G. Van Der Kraak, 1988. Induced ovulation and spawning in cultured fresh water fish in China: advanced in application of GnRH analogue and dopamine antagonists.Aquaculture, 74: 1-10.
11. Kelly, A.M. and C.C. Kohler, 1994. Human chorionic gonadotropin injected in fish degrades metabolically and by cooking. J. the World Aquaculture Society, 25: 55-57.
12. Park, I.S., E.Y. Chung and K.P. Hong, 1997. Hormonal induction of ovulation in the coho salmon, *Oncorhynchus kisutch*. J. Aquaculture, 10: 485-486.
13. Zohar, Y., 1989. Fish reproduction, its physiology and artificial manipulation. In: M.C. Shilo and S.H. Sargi, (Eds.), Fish culture in warm Water System, Problems and Trends. CRC Press, pp: 65-119.
14. Zohar, Y. and C.C. Mylonas, 2001. Endocrine manipulation of spawning induction in cultured fish from hormone to gene. Aquaculture, 197: 99-139.
15. Syndel International Inc., 2003. Using Ovaprim to induce spawning in cultured fish. http:// www.syndel.com/spawning/using_ovaprim.html.
16. Drori, S., M. Ofir, B.L. Sivan and Z. Yaron, 1994. Spawning inductionin common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with methoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence ontemperature. Aquaculture, 119: 393-407.
17. Glasser, F., T. Mikolajczyk, B. Jalabert, J. Baroiller and B. Berton, 2004. Temperature effects along the reproductive axis during spawning induction of grass carp (Ctenopharyngodon idella). General and Comparative Endocrinol., 136: 171-179.

18. Rutaisire, J. and A.J. Booth, 2004. Induced ovulation, spawning, egg incubation and hatching of the cyprinid fish *Labeo victorianus* in captivity. J. the World Aquaculture Society, 35: 383-391.
19. Hill, J.E., J.D. Baldwin, J.S. Graves, R. Leonard, G.F.F Powell and C.A Wanton, 2005. Preliminary observations of topical gill application of reproductive hormones for induced spawning of a tropical ornamental fish. North American J. Aquaculture, 67: 7-9.
20. Sahoo, S.K., S.S. Giri, S. Chandra and A.K. Sahu, 2007. Effect of Ovaprim doses and latency period on induced spawning of Clarias batrachus: Observation on larval deformity. Indian J. Experiment Biol., 45: 920-922.
21. Bromage, N.R. and P.R.T. Cumaranatunga, 1988. Egg production in rainbow trout. In Recent Advances in Aquaculture, Eds., J.F. Muir and R.J. Roberts Croom Helm., London, pp: 65-138.
22. Mylonas, C.C., J.M. Hinshaw and C.V. Sullivan, 1992. GnRHa induced ovulation of brown trout (Salmo trutta) and its effects on egg quality. Aquaculture, 106: 379-392.
23. Kulikovsky, Z., F.J.B. Martin and Z. Yaron, 1996. A comparison of two spawning inducing agent for common carp. Isr. J. Aquac. Bamidgeh, 48: 108-111.
24. Billard, R., 1990. The major carps and other cyprinids. In world Animal Sciences CIIX, Production of Aquatic Animals (Fishes), Ed., Nash, C.E. Elsevier Science Publication, pp: 21-55.
25. Szabo, T., C. Medgyasszay and L Horvath, 2002. Ovulation induction in nase (*Chondrostoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with Domperidone, Aquaculture, 203: 389-395.
26. Weil, C., A. Fostier and R. Billard, 1986. Induced spawning (ovulation & spermiation) in carp & related species. In Aquaculture of Cyprinids Billard, Eds., R. Marcel and J. Inra, Paris, pp: 119-137.
27. Leelapatra, W., 1988. Carp culture in Thailand withparticular emphasis on induced spawning. In Proceedings of the Aquaculture International Congress and Exposition, Vancouver, B.C., 6-9 September, pp: 331-337.
28. Kumarasini, W.S.A. and P. Seneviratne, 1988. Induced multiple spawning of Chinese carps in Srilanka. Aquculture, 74: 57-62.
29. Chaudhuri, H., 1976. Use hormones in induced spawning of carps, J. Fish Res. Boord Can., 33: 940-947.
30. Thalathiah, S., A.O. Ahmad and M.S. Zaini, 1988. Induced spawning techniques practiced at Batu Berendam, Malaka. Aquaculture, 74: 23-33.
31. Nandeesha, M.C., K.G. Rao, R. Jayanna, N.C. Parker, T.J. Varghese, P. Keshavanathand and H.P.C. Shetty 1990. Induced spawning of Indian major carps throughsingle application of Ovaprim. In: Proceedings of The Second Asian Fisheries Forum, Eds., R. Hirano and I. Hanyu. Asian Fisheries Society, Manila, Philippines, pp: 581-585.
32. Das, S.K., B.K. Bhattacharjya and K. Sarma, 1994. Induced spawning and hatching of Tawes, Puntius javanicus (Bleek er). Asian Fisheries Science. Philippines, 7: 191-194.
33. Sahoo, S.K., S.S. Giri and A.K. Sahu, 2005. Effect on Breeding Performance and egg Quality of Clarias batrachus (Linn.) at various Doses of Ovatide During Spawning Induction. Asian Fisheries Sci., 18: 77-83.
34. Zarski, D., K. Targonska, S. Ratajski, Z. Kaczkowski and D. Kucharczyk, 2008. Reproduction of Nase, *Chondrostoma nasus* (L.), under controlled condition. Archives of Polish Fisheries, 16: 355-362.
35. Yamazaki, F. and E.M. Donaldson, 1968. The effects of partially purified salmon pituitary gonadotropin on spermatogenesis, vitellogenesis and ovulation in hypophysectomized goldfish, *Carassius auratus*. Gen. Comp. Endocrinol., 11: 292-299.
36. Bhowmick, R.M., 1979. Observations on the human chorionic gonadotropin prepared in inducing spawning in major carp *Labeo rohita.* Ham. Tech., pp: 18.
37. Dave, H.S. and T. Sukumaran, 1984. Some observations on the use of human chorionic gonadotropin for induced breeding of Indian and Chinese carps. In: Souvenir of tthe Seminar on Freshwater Fisheries and Rural Development, Rourkela (Orissa), pp: 126.
38. Chonder, S.L., 1986. Repeated breeding of Indian and Chinese major carps during the spawning season. Induced breeding of carps. CIFRI, Barrackpore, pp: 40-43.
39. Van Der Kraak, G., J.P. Chang and D.M. Janz, 1988. Reproduction. In: D.H. Evans, (ed): The Physiology of Fishes. CRC Press LLC, Florida, USA., pp: 465-488.
40. Harvey, B., J. Nacario, L.W. Crim, J.V. Juario and C.L. Marte, 1985. Induced spawning of sea bass, *Lates calcarifer* and rabbitfish, *Siganus guttatus*, after implantation of pelleted LHRH analogue. Aquaculture, 47(1): 53-59.

41. Marte, C.L., N.M. Sherwood, L.W. Crim and B. Harvey, 1987. Induced spawning of maturing milkfish (Chanos chanos Forsskal (With gonadotropin-releasing-hormone (GnRH) analogues administered in various ways. Aquaculture, 60(3): 301-303.
42. Mortezavizadeh, S.A., M. Yooneszadeh Feshalami and F. Bosak Kahkesh, 2010. Effect of GnRHa (Ala6,des-Gly10 mGnRHa), LHRH-a(des-Gly10,[D-ala6]LH-LH Ethylamide and Carp Pituitary in Artificial Propagation of Gattan, *Barbus xanthopetrus* (Heckel, 1843). World J. Fish and Marine Sci., 2(4): 280-284.
43. Alok, D., T. Krishnan, G.P. Talwar and L.C. Grag, 1993. Induced spawning of catfish, Heteropneustes fossilis (Bloch), using D-Lys6 salmon gonadotropin releasing hormone analogue. Aquaculture, 115: 159-167.
44. Haniffa, M.A.K. and S. Sridhar, 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionicgonadotropin and synthetic hormone (ovaprim). Veterinarski arhiv, 72(1): 51-56.
45. Yaron, Z., 1995. Endocrine control of gametogenesis and spawning induction in carp. Aquculture, 129: 49-73.
46. Dorafshan, S., H. Mostafavi and B.M. Amiri, 2003. Induction of spawning in common carp (*Cyprinus carpio*) using pituitaryextract and GnRH analogue in combination with Domperidone.Iran. J. Biotechnol., 1: 213-217.
47. Arabaci, M. and M. Sari, 2004. Induction of ovulation in endemic pearl mullet (*Chalcalburnus tarichi*), living in the highly alkaline Lake Van, using GnRHa ([D-Ser (tBu)6, Pro9-Net]-GnRH) combined with haloperidol. Aquaculture, 238: 529-535.
48. Ngamvongchon, S., O. Pawaputanon, W. Leelapatra and W.E. Jonson, 1988. Effectiveness of LHRH analogous for the Induced spawning of carp and catfish in Northeast Thailand. Aquculture, 74: 35-40.
49. Heyrati, F.P., H. Mostafavi, H. Toloee and H. Dorafshan, 2007. Induced spawning of kutum (Kamenskii, 1901) using (D-Ala^6, Pro^9 -Net) GnRHa Combined with Domperidone. Aquculture, 265: 288-293.