

ARTIFICIAL SPAWNING OF COMMON CARP (*CYPRINUS CARPIO*), GRASS CARP (*CTENOPHARYNGODON IDELLA*) AND SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) INDUCED PITUITARY EXTRACT OF CARP AND OVAPRIM HORMONE

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ABSTRACT

Studies have shown that final gamete maturation in common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) can be induced by the administration of carp pituitary extract (CPE) and Ovaprim (O). A total number of 15 females and 15 males common carp (*Cyprinus carpio*), 15 females and 15 males grass carp and 15 females and 15 males silver carp were divided into three groups. The first group (T1) was injected with CPE (double dose). The second group (T2) was injected with CPE (one dose) and the third group (T3) was injected with Ovaprim 0.5 mg/kg one dose. Latency time, mass of eggs, egg weight index percentage (EWIP%), ovulation rate, fertilization and hatching rate, cost of injection, return efficiency and economic efficiency were recorded. The results in Common carp (*Cyprinus carpio*) showed that latency time in T1 was 10 hrs, whereas T2 and T3 was 16 and 14 hrs, respectively. While, spawning rate in three groups were 80, 65 and 90% of females were spawned in T1, T2 and T3, respectively, and mass of eggs; EWIP % and return were significantly higher in T1 than in T3 and T2. However, cost was higher significantly in T1 than in T2 and T3. The fertilization rate was higher significantly in T2 than in both T1 and T3, but differences between T2 and 1 was insignificantly. Also, the hatching rate was higher significantly in T2 (77±4.5 %) than in both T1 and T3. While, the results in grass carp (*Ctenopharyngodon idella*) showed that latency time of T1 was 8 hours, whereas, T2 and T3 were 12 and 10 hrs respectively. Also, spawning rate in groups were 90, 50 and 80 % of females were spawned in T1, T2 and T3, respectively, and mass of eggs (476±57 g) and return (3628.6 ± 486.4 LE) was significantly higher in T1 than in both T2 and T3, respectively. While, differences of EWIP % between the T1, T2 and T3 were insignificantly. The mean fertilization percentage (79±6.5 %), hatching rate (74±6.5 %) and cost (27.6±9.6 LE) were higher significantly in T1 than in T2, but the difference between T1 and T3 was insignificantly. On the other hand, the results in silver carp (*Hypophthalmichthys molitrix*) showed that, Latency time of T1 was 8 hours; whereas, T2 & T3 were 13 and 9 hours, respectively. While, spawning rate in three groups were 90, 50 and 80 % of females were spawned in T1, T2 and T3, respectively, and mass of eggs (310±54.7 g); fertilization rate % (81±4.2 %); hatching rate (76 ± 4.2 %); cost (30.8±3.5 LE) and return (2787.1±475.4 LE) in T1 than in T2 and T3. Also, EWIP % was higher significantly in T3 than in T1 and T2. On the other

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hand, the economic efficiency was insignificantly in the three groups, while, the results of the economic efficiency was high in T2 than in T1 and T3.

Key words: Reproductive performances; Carp pituitary extract; Ovaprim, Common carp (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitrix*).

INTRODUCTION

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances (Rottmann, *et al.*, 1992). Many fish spawn in environment that are nearly impossible to simulate in a hatchery, therefore, hormone-induced spawning is the only reliable method to induce reproduction in these fishes. One of the most commonly applied spawning agents is carp pituitary extract (Akar, 2006, 2008 and Akar and Ali, 2006). The pituitary gland produces and stores gonadotropin hormones (Gth), which play a decisive role in ovulation and spermatation. Injected pituitary material by passes the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood (Gth) levels that normally precedes spawning (Rottmann, *et al.*, 1992).The efficacy of common carp pituitary to induce ovulation and spermatation in fish is well documented (Brzuska and Bialowas, 2002 and Akar, 2006). Not only was carp pituitary injection one of the very first methods of inducing ovulation, and spermatation in fish, it has stood the test of time and is still the preferred methodology of many fish culturists. In some situations, it has been found to be the most efficient and reliable method of inducing final gamete maturation (Erdahl, 1996).

A recent development in the technology of induced breeding is the stimulation of endogenous gonadotropin (Gth) release from the pituitary of the treated fish using a synthetic analogue of gonadotropin releasing hormone (GnRH α). Because certain analogues are inexpensive and effective, this method is gaining acceptance throughout the world. GnRH α treatments have been found to induce and synchronize ovulation in several commercially important fresh water and marine species Szabo, 2003 .On the other hand, the increasing of the cyprinid culture in the world caused the problem in the presenting of calibrated CPE to aquaculturists. This

led to the development of a new approach in the inducing of spawning for cyprinid fish. In this approach, different ovaprim forms and their analogues, stimulating of endogenous Gth release, are used with a dopamine receptor antagonist (DA), which potentiates response to the peptide (Peter *et al.*, 1988 and 1992). The simple method of treating fish with complex substances (Ovaprim) containing (Peter *et al.* 1992 and Brzuska and Adamek 1999). Ovaprim is one of these substances, satisfactory results have been obtained using Ovaprim as an ovulation stimulator in Carp (Brzuska and Adamek 1987 and Amer *et al.* 2009), in African catfish females (Akar 2008), in European catfish females (Brzuska and Adamek 1999) , in Northern pike (Szabo 2003) and in Nase (Szabo *et al.* 2002).

Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary's positive response to injected LHRHa. There is a family of drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for induce spawning (Drori *et al.* 1994; Brzuska, 1999; Dorafshan *et al.*, 2003 and Arabaci *et. al.* 2004).

The objectives of the present study to examine the effects of Ovaprim on induction of spawning in common carp, grass carp and silver carp. Also, compare two methods of induce spawning by carp pituitary extract (CPE) either in one dose or in two successive doses for three species intra-peritoneal

MATERIAL AND METHODS

A total number of 90 brood fish contained 15 females (2.368-3.0109 kg/fish) and 15 males of common carp, 15 females (2.509-4.2709 kg/fish) and 15 males of grass carp and 15 females (2.800-4.040 kg/fish) and 15 males of silver carp were selected from over-wintered carp in earthen ponds of Central laboratory for Aquaculture Research, Abbassa, Abu-Hamad, Sharkia, Egypt. Fish were divided into three groups. The first treatment was injected with CPE (double dose) at intra-peritoneal and dose 2 injected after 8 hours from dose 1 (stimulation dose). The second treatment was injected intra-peritoneal with CPE (one dose) and the third treatment was injected with Ovaprim 0.5 mg/kg one dose intra-peritoneal. On the late days of April, the spawners were caught, after selection, sexing and transferred

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to a quarter feddan with 1 meter depth. At the spawning season, female carp which showed spawning signs were randomly divided and distributed in nine tanks of 3.5 m volume filled with filtered Nile water supplied with aerator. Each tank contained ten individuals.

After acclimatization of fish for 24 hrs the water quality was measured as table 1.

Table1: Physico-chemical characteristics of earthen water ponds of Common carp (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitrix*) during climatic period before the experimental.

Items	Mean	Items	Mean
Temperature(c)	23-25 °C	Nitrate (mg/l)	0.01
PH	8.7	Nitrite (mg/l)	0.02
Oxygen (mg/l)	8.1	Salinity (mg/l)	0.3

Salinity was calculated by relation (1000 micromos = 0.7g salinity according to Dewis and Freila, 1970).

Ovulation ratio and EWIP were estimated according to Szabo (2003)

Ovulation ratio = number of ovulated females /number injected.

Egg weight index percentage (EWIP) = (weight of stripped egg mass/BW before stripping) *100

For eggs of both experiments, the percentage of fertilization was estimated after 6 hrs of incubation according to Gheyas, *et al.* (2001) as follows:

Fertilization rate = (Number of fertilized eggs / Total number of eggs) * 100

Hatching rate = (Number of hatched eggs (larvae) /Total number of eggs) *100

Then after 24 hrs of incubation hatching percentage was estimated.

Statistical analysis all statistics were carried out using statistical analysis systems (SAS, 2004).

RESULTS AND DISCUSSION

Common carp:

Common carp was induced after 10 hours from injected with double dose of CPE. While, it rallies in fish after injected with one dose of CPE and ovaprim were accorded after 16 and 14 hours, respectively (table 2). Spawning rate in females groups of common carp were 80, 65 and 90% of spawned in treatment T1, T2 and T3, respectively. These results were in agreement with the findings of Kouril *et al.*

(2003) showed that the higher percentage of females' tench (*Tinca tinca*) spawned after lecrelin treatment than CPE (89 % and 23 % respectively).

The results of (Table 2 and Figs 1-4) showed that the mass of eggs (360 ± 44.2 g); EWIP % (11 ± 1.5 %) and return (1311.8 ± 138.4 LE) were significantly higher in group 1 than in T3 (284 ± 47.7 g; 9.8 ± 2.1 % & 1036.2 ± 209 LE, respectively) and T2 (198 ± 47.1 g; 7.2 ± 1.9 % & 767.4 ± 146.7 LE, respectively). However, the cost was higher significantly in T1 (1311.8 ± 138.4 LE) than in T2 (767.4 ± 146.7 LE) and T3 (1036.2 ± 209 LE). The fertilization rate was higher significantly in T2 (82 ± 4.5 %) than in T1 (76 ± 2.2 %) and T3 (63 ± 13.5 %), but differences between T2 and 1 was insignificantly. Also, the hatching rate was higher significantly in T2 (77 ± 4.5 %) than in T1 (70 ± 3.5 %) and treatment 3 (60 ± 12.3 %). The present results are in agreement with Mahmood (2006) who showed that splitting of the CPE dose (double dose) did not affect the ovulation time and ovulation response in climbing perch (*Anabas testudineus*). On the other hand, the economic efficiency was higher significantly ($P < 0.05$) in T1 (5209.1 ± 472.2 %) than in T3 (3687.7 ± 667.4 %).

Table 2: Effect of carp pituitary extract and ovaprim hormone on hatching parameters of common carp (*Cyprinus carpio*) treatments.

Investigation	Carp pituitary extract double dose (T1)	Carp pituitary extract one dose (T2)	Ovaprim (T3)
Latency time (hours)	10	16	14
Body weight before spawning (g)	3010 ± 347.0^a	2368 ± 670.8^a	2748 ± 646.8^a
Body weight after spawning (g)	2650 ± 331.6^a	2170 ± 544.9^a	2460 ± 559.0^a
Mass of eggs (g)	360 ± 44.2^a	198 ± 47.1^c	284 ± 47.7^b
EWIP (%)	11.0 ± 1.5^a	7.5 ± 1.9^b	9.8 ± 2.1^{ab}
Fertilization rate (%)	76 ± 2.2^a	82 ± 4.5^a	63 ± 13.5^b
Hatching rate (%)	70 ± 3.5^{ab}	77 ± 4.5^a	60 ± 12.3^b
Cost	25.2 ± 1.6^a	15.6 ± 3.3^b	27.2 ± 6.3^a
Return	1311.8 ± 138.4^a	767.4 ± 146.7^c	1036.2 ± 209.0^b
Economic	5209.1 ± 472.2^a	4377.7 ± 748.9^{ab}	3687.7 ± 667.4^b

Grass carp:

Grass carp was induced after 8 hours from injected with double dose of CPE. While, it rallies in fish after injected with one dose of CPE and ovaprim were accorded after 12 and 10 hours, respectively (table 3). Spawning rate in females groups of grass carp were 90, 50 and 80% of spawned in treatment T1, T2 and T3, respectively. These results were contrary with the findings of Brzuska (2003).

The results of (Table 3 and Figs 5-8) showed that the mass of eggs (420 ± 57 g) and return (3628.6 ± 486.4 LE) was significantly higher in group 1 than in group 2 (200 ± 50 g & 1693.8 ± 407.4 LE respectively) and group 3 (280 ± 25.5 g & 2425.2 ± 145.7 LE, respectively). While, differences of EWIP % between the group T1 (10.3 ± 3.6 %), T2 (7.9 ± 1.6 %) and T3 (10.3 ± 0.6 %) were non significantly. These results agreement with findings Brzuska (2003) and Akar and Ali (2006). The increase in the ovulation of the Common carp Grass carp may be due to that the Oogenesis is controlled by stimulating hormone (FSH) and Luitnasing hormone (LH) but need also participation of several paracrine autocrine mechanisms of regulation as reported by Koural *et al.* (2003).

Table 3: Effect of carp pituitary extract and ovaprim hormone on hatching parameters of grass carp (*Ctenopharyngodon idella*) treatments.

Investigation	Carp pituitary extract double dose (T1)	Carp pituitary extract one dose (T2)	Ovaprim (T3)
Latency time (hours)	8	12	10
Body weight before spawning (g)	4270 ± 912.1^a	2504 ± 166.8^b	2720 ± 389.4^b
Body weight after spawning (g)	3850 ± 842.0^a	2500 ± 583.1^b	2440 ± 364.7^b
Mass of eggs (g)	420 ± 57.0^a	200 ± 50.0^c	280 ± 25.5^b
EWIP (%)	10.3 ± 3.6^a	7.9 ± 1.6^a	10.3 ± 0.6^a
Fertilization rate (%)	79 ± 6.5^a	63 ± 6.7^b	78 ± 2.7^a
Hatching rate (%)	74 ± 6.5^a	59 ± 16.3^b	73 ± 2.7^a
Cost	27.6 ± 9.6^a	16.0 ± 1.4^b	26.8 ± 4.4^a
Return	3628.6 ± 486.4^a	1693.8 ± 407.4^c	2425.2 ± 145.7^b
Economic	13590.7 ± 3176.7^a	10941.0 ± 1305.1^{ab}	9186.6 ± 1067.9^b

The mean fertilization percentage (79 ± 6.5 %), hatching rate (74 ± 6.5 %) and cost (27.6 ± 9.6 LE) were higher significantly in group 1 than in group 2 (63 ± 6.7 %, 59 ± 16.3 % & 16 ± 1.4 LE, respectively), While these were insignificant differences between T1 and T3 in fertilization and hatching rate. The results may be relation to the negative effect of the female hormones on the male carp, this attribution need further investigation. The present results are in agreement with Brzuska (2004) and Akar (2006). On the other hand, the economic efficiency was higher significantly ($P < 0.05$) in group 1 (13590.7 ± 3176.7 %) than in group 3 (9186.6 ± 1067.9 %), but group 2 (10941 ± 1305.1 %) was between group 1 and 3.

Silver carp:

Silver carp was induced after 8 hours from injected with double dose of CPE. While, it rallies in fish after injected with one dose of CPE and ovaprim were accorded after 13 and 9 hours, respectively (table 4). Spawning rate in females groups of silver carp were 90, 50 and 80% of spawned in treatment T1, T2 and T3, respectively. These results were contrary with the findings of Brzuska (2004) and Akar (2006) showed that synchronization of ovulation silver carp was observed in all the females after the injection by Aquaspawn.

Table 4: Effect of carp pituitary extract and ovaprim hormone on hatching parameters of silver carp (*Hypophthalmichthys molitrix*) treatments.

Investigation	Carp pituitary extract double dose (T1)	Carp pituitary extract one dose (T2)	Ovaprim (T3)
Latency time (hours)	8	13	9
Body weight before spawning (g)	4040 ± 616.8^a	3520 ± 907.3^b	2800 ± 280.6^b
Body weight after spawning (g)	3730 ± 569.6^a	3260 ± 847.4^{ab}	2540 ± 270.2^a
Mass of eggs (g)	310 ± 54.7^a	260 ± 65.2^a	260 ± 41.8^a
EWIP (%)	7.6 ± 0.7^b	7.4 ± 0.7^b	9.3 ± 1.5^a
Fertilization rate (%)	81 ± 4.2^a	78 ± 4.5^a	79 ± 4.2^a
Hatching rate (%)	76 ± 4.2^a	73 ± 4.5^a	74 ± 4.2^a
Cost	30.8 ± 3.5^a	21.6 ± 6.2^b	28.2 ± 2.2^a
Return	2787.1 ± 475.4^a	2234.6 ± 473.5^a	2266.8 ± 255.8^a
Economic	9038.8 ± 1009.6^a	16649.3 ± 12327.5^a	8074.7 ± 1039.3^a

The results of (Table 4 and Fig 9-12) showed that the higher levels in the

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mass of eggs (310 ± 54.7 g); fertilization rate % (81 ± 4.2 %); hatching rate (76 ± 4.2 %); cost (30.8 ± 3.5 LE) and return (2787.1 ± 475.4 LE) in group 1 than in group 2 (260 ± 65.2 %, 78 ± 4.5 %, 73 ± 4.5 %, 21.6 ± 6.2 and 2234.6 ± 473.5 LE, respectively) and group 3 (260 ± 41.8 %, 79 ± 4.2 %, 74 ± 4.2 %, 28.2 ± 2.2 and 2266.8 ± 255.8 LE, respectively) but the differences between them insignificantly. While, EWIP % was higher significantly in group 3 (9.3 ± 1.5 %) than in group 1 (7.6 ± 0.7 %) and group 2 (7.4 ± 0.7 %). On the other hand, the economic efficiency was insignificantly in the three groups, while, the results of the economic efficiency was high in group 2 (16649.3 ± 12327.5 %) than in group 1 (9038.8 ± 1009.6 %) and group 3 (8074.7 ± 1039.3 %). The increase in the ovulation of the Common carp and Grass carp may be due to that the Oogenesis is controlled by stimulating hormone (FSH) and Luitnasing hormone (LH) but need also participation of several paracrine autocrine mechanisms of regulation as reported by Kourai *et al*, (2003). Also the increase in ovulation may be due to that the HCG is increase to speed the maturation of the eggs in fish as reported by Hodson and Sullivan (1993).

From the previous results could be concluded that the injection of injected with CPE (double dose) at intra-peritoneal and dose 2 injected after 8 hours from dose 1 (stimulation dose) was the most effective treatment in economic efficiency possessed the most effective results in the artificial spawning of common carp; grass carp and silver carp.

Fig (1): Effect of carp pituitary extract and ovaprim hormone on egg weight of common carp (*Cyprinus Carpio* L.).

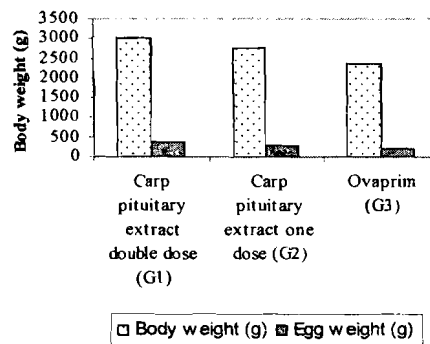


Fig (2): Effect of carp pituitary extract and ovaprim hormone on EWIP % of common carp (*Cyprinus Carpio* L.).

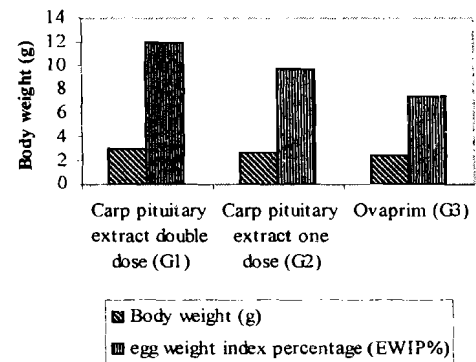


Fig (3): Effect of carp pituitary extract and ovaprim hormone on fertility and hatchability % of common carp (*Cyprinus Carpio L.*).

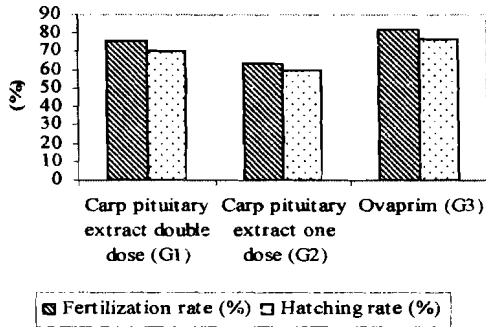


Fig (4): Effect of carp pituitary extract and ovaprim hormone on Return (LE) and Economic efficiency of common carp (*Cyprinus Carpio L.*).

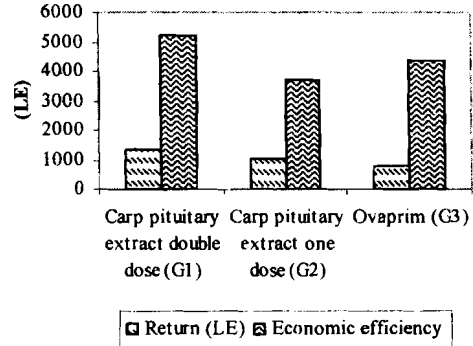


Fig (5): Effect of carp pituitary extract and ovaprim hormone on egg weight of grass carp (*Ctenopharyngodon idella*).

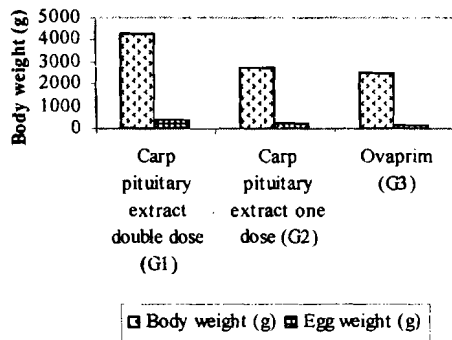


Fig (6): Effect of carp pituitary extract and ovaprim hormone on EWIP% of grass carp (*Ctenopharyngodon idella*).

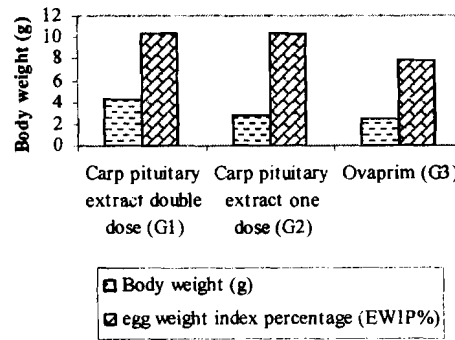


Fig (7): Effect of carp pituitary extract and ovaprim hormone on fertility and hatchability % of grass carp (*Ctenopharyngodon idella*).

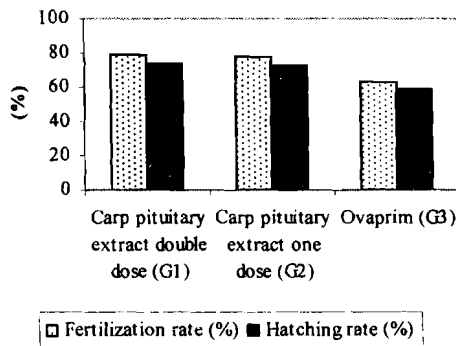
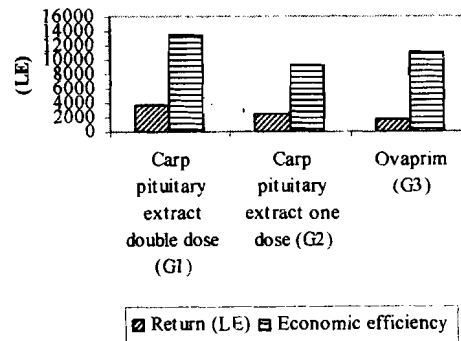


Fig (8): Effect of carp pituitary extract and ovaprim hormone on Return (LE) and Economic efficiency of grass carp (*Ctenopharyngodon idella*).



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Fig (9): Effect of carp pituitary extract and ovaprim hormone on egg weight of silver carp (*Hypophthalmichthys molitrix*).

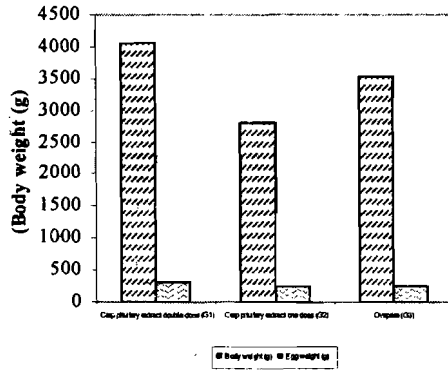


Fig (10): Effect of carp pituitary extract and ovaprim hormone on EWIP % of silver carp (*Hypophthalmichthys molitrix*).

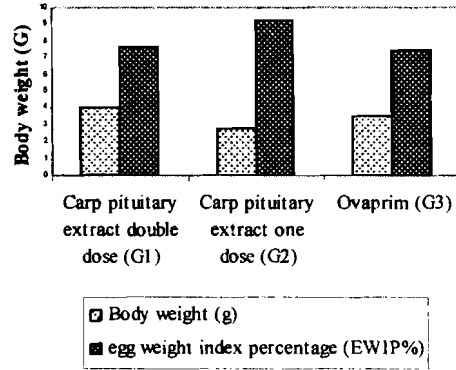


Fig (12): Effect of carp pituitary extract and ovaprim hormone on Return (LE) and Economic efficiency of silver carp (*Hypophthalmichthys molitrix*).

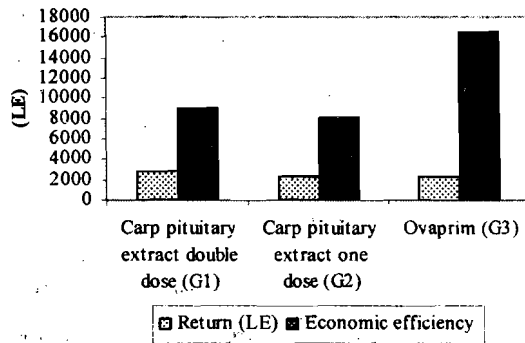
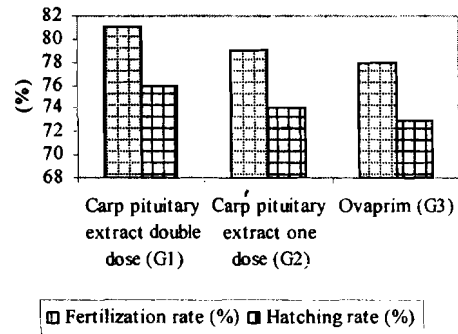


Fig (11): Effect of carp pituitary extract and ovaprim hormone on fertility and hatchability % of silver carp (*Hypophthalmichthys molitrix*).



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التفريخ الصناعي للمبروك العادى والحشائش والفضى بالحقن بمستخلص الغدة

النخامية وهرمون الافبريم

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المعمل المركزي لبحوث الثروة السمكية بالعباسة

استهدفت هذه الدراسة تأثير الحقن بمستخلص الغدة النخامية (كجرعه واحدة أو جرعتين) وهرمون الافبريم علي الكفاءة التناسلية للمبروك العادى والحشائش و الفضى واستخدم هذا البحث ٣٠ سمكة مبروك عادى ١٥ ذكور و ١٥ إناث و ٣٠ سمكة مبروك حشائش ١٥ ذكور و ١٥ إناث و ٣٠ سمكة مبروك فضى ١٥ ذكور و ١٥ إناث وقسمت الاسماك الى ٣ مجموعات. المجموعة الاولى تم حقنها بالغدة النخامية علي جرعتين والمجموعة الثانية حقنت بجرعة واحدة والمجموعة الثالثة حقنت بهرمون الافبريم وتم دراسة الزمن بين الحقنة الأولى والتبويض وكمية البيض ودليل المناسل فى الثلاث انواع من المبروك ونسبة الإخصاب ونسبة الفقس والعائد والكفاءة الاقتصادية وكانت النتائج كما يلي:

أولاً - المبروك العادى:

- الزمن بين الحقنة الأولى والتبويض كان (١٠ ساعات) أسرع وأفضل في المجموعة الأولى ونسبة التبويض ٨٠% فى المجموعة الاولى و ٦٥% فى المجموعة الثانية و ٩٠% فى المجموعة الثالثة وحدثت زيادة معنوية لكمية البيض ودليل المناسل والعائد في المجموعة الاولى عن المجموعة الثالثة والثانية وزيادة فى التكلفة فى المجموعة الاولى عن باقى المجموعات وحدثت زيادة معنوية في نسبة الإخصاب والفقس في المجموعة الثانية عن المجموعة الاولى والثالثة.

ثانياً - مبروك الحشائش:

- الزمن بين الحقنة الأولى والتبويض كان (٨ ساعات) أسرع وأفضل في المجموعة الأولى ونسبة التبويض ٩٠% فى المجموعة الاولى و ٥٥% فى المجموعة الثانية و ٨٠% فى المجموعة الثالثة وحدثت زيادة معنوية لكمية البيض ٤٢٠ جم والعائد ٣٦٢٨ جنية فى المجموعة الاولى عن المجموعة الثانية والثالثة ولا يوجد فرق معنوى بين المجموعات الثلاثة فى دليل المناسل وحدثت زيادة معنوية في نسبة الإخصاب والفقس في المجموعة الاولى عن الثانية ولا فرق معنوى بين المجموعة الاولى والثالثة.

ثالثاً - المبروك الفضى:

- الزمن بين الحقنة الأولى والتبويض كان (٨ ساعات) أسرع وأفضل في المجموعة الأولى ونسبة التبويض ٩٠% فى المجموعة الاولى و ٥٠% فى المجموعة الثانية و ٨٠% فى

المجموعة الثالثة وحدثت زيادة معنوية لكمية البيض ٣١٠ جم ونسبة التلقيح ٨١ % والفقس ٧٦ % والتكلفة ٣٠,٨ جنية والعائد ٢٧٨٧,٠ جنية في المجموعة الأولى عن المجموعة الثانية والثالثه ودليل المناسل اعلى معنوية في المجموعة الثالثة عن المجموعة الأولى والثانية والكفاءة الاقتصادية اعلى للمجموعة الثانية عن باقي المجموعات. مما سبق نوصي باستخدام من مستخلص الغدة النخامية بالحقن على جرعتين عند تفريخ المبروك العادي والحشائش.