EFFECT OF SEASONAL CHANGES AND HORMONAL INJECTION ON SOME BIOCHEMICAL PARAMETERS AND SOME MILT PROPERTIES IN MALE COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

Males of common carp (*Cyprinus carpio*) with average weight 1500±45g were collected from the fish farm of the Central Laboratory for Aquaculture Research (CLAR) Abbassa Abou Hamad Sharkia Egypt. 144 males were divided into four groups, first control (T1), second treatment (T2) males injected with pituitary extract, third treatment (T3) males injected with human chronic gonadotropin (hCG) and fourth treatment (T4) males injected with both pituitary extract and hCG. Semen was collected repeatedly from all groups of common carp and the effect of pituitary extract, HCG hormones injection on various sperm parameters was investigated in autumn 2006 and spring 2007. Seasonal differences in sperm characteristics were observed from spring to autumn. Highly significant positive relationship existed between biochemical parameters before and after Anaesthesia with MS222 in the same season. A significant increase in means of acetylcholine esterase (Ach E.), cortisol hormone and sugar levels occurred before and after Anaesthesia throughout within the same season but the activity of serum total protein, albumin and total globulin variability explained by collection date was low due to variability between males. Water quality was measured before spawning season in spring or autumn, by measuring temperature, dissolved oxygen, ammonia and salinity. Different zooplankton orders were investigated during summer and winter also.

Key words: Anaesthesia, biochemical parameters, (Ach E., cortisol hormone and sugar), hormonal injection, semen, milt volume, recovery time, and male common carp.

INTRODUCTION

The handling of aquatic animals both in and out of their natural environment almost always involves physical activity. Their characteristic struggling during capture and handling affects physiology and behavior, and consequently, it is often necessary to immobilize fish before attempting to perform even the simplest task (Ross and Ross, 1999). The temperature of water and photoperiod are the environmental conditions which have the strongest impact on maturation of fish gametes (Kujawa, *et al.* 1999). Artificial spawning and obtaining good quality gametes is one of the most important problems in aquaculture. Generally, these problems are connected with reproduction of wild, especially cyprinid fish species, mostly endangered, which have regional importance for aquaculture or angling (Kucharczyk *et al.*, 1997).

The neuroendocrine and immune systems are intimately linked and involved in bi-directional communication, which performs a homeostatic function (Berczi and Nagy, 1998). Steroid hormones not only affect the specific immune responses, but also the non-specific defense system (Magnusson and Fossum 1992). Stress-related cortisol releases in fish can suppress immunological capacity and affect of seawater tolerance (Redding and Schreck, 1983). Anesthetics are therefore useful to reduce or minimize stress to the fish. The choice of anesthetics generally depends on several considerations: (1) availability; (2) cost-effectiveness; (3) ease of use; (4) nature of the study; and (5) safety for the user (Cho and Heath, 2000).

The initiation of the heart beat and its abnormalities are very important issues in the field of cardiac physiology and pathoelectrophysiology. Abnormalities of cardiac impulse initiation are usually considered as belonging to either automatic or triggered rhythms (Waldo 1993). In the past two decades there has been renewed interest in atrial re-entrant rhythms, such as atrial flutter and fibrillation (Janse and Allessie 1992).

Sperm quality is a measure of the ability of sperm to fertilize an egg successfully, but this capacity may not be reliable, as egg quality may be variable

and affect fertilization success (Rurangwa *et al.,* 2004). Also they added that any quantifiable physical parameter that directly correlates with the fertilization capacity of sperm could be used to evaluate sperm quality. The percentage of motile spermatozoa is the most common test used to evaluate fish sperm quality (He and Woods 2004). Usually, captive males from different species produce small amounts of milt with low sperm, and hormonal treatments have been normally employed to solve these problems (Mylonas and Zohar 2001). Among gonadotropin preparations, HCG is the most commonly used one because of its wider availability, higher purity and standardized activity (Donaldson 1996); consequently, HCG has been used in spawning induction of many culture fish species (Pankhurst and Poortenaar, 2000). Commercially available carp and salmon pituitary extracts also increased sperm release in pejerrey. Similar results were obtained for different fish species using homologous pituitary extracts, ranging from 2 to 10 mg kg^{-1} (Parauka, *et al.*, 1991).

As zooplankton differs in their preference for biotic and abiotic factors, some species are suggested to be good indicators of lake trophic status (Attayde & Bozelli, 1998). Therefore, the seasonal temperature changes may be another reason responsible for the shift of crustacean community (Tackx *et al.*, 2004).

The aim of the present study was to examine the effect of seasonal changes of water quality and hormonal injection (pituitary extract, HCG and their mixture treatments) on zooplankton community in earthen ponds in summer and winter, spermatozoa quality of common carp and serum Ach. E, cortisol and sugar levels before and after using MS222 as Anaesthesia in autumn and spring.

MATERIALS AND METHODS

One hundred and forty four (144) males of common carp with average weight 1500±45g were collected from the fish farm of the Central Laboratory for Aquaculture Research (CLAR) Abbassa Abou Hamad Sharkia Egypt. Three months before the beginning of the spawning seasons (autumn and spring) males collected

and stock in earthen ponds with 1000 m^2 in area and feed on 25% protein at 3% of body weight two times daily. Water samples were collected biweekly from three earthen ponds for water analysis and zooplankton examination and means were tabulated monthly. At the end of this period males collected and transported to circular fiber glass with $10m^2$ in surface area for 24 hour to remove the stress due to harvest, water change and transport. They were divided into four groups, each group has three replicates; each replicate has six fish, the first was control group (T1), the males of second group were injected with pituitary extract (T2), the third males group injected with human chronic gonadotropin (HCG) (T3) and the fourth males group injected with pituitary extract and HCG (T4) for two seasons (autumn and spring). Semen was collected repeatedly from all groups of common carp and the effect of pituitary extract, HCG hormones injection on various sperm parameters was investigated in autumn 2006 and spring 2007 with and without using MS222. In all treatments, T2 pituitary extract (3mg/male), T3 HCG (1000 IU/male), T4 (pituitary extract 1.5mg/male and 500IU/male). After injection by 8 hours all samples of semen and blood were collected.

A- Water quality

A-1-Water analysis

Water samples were collected from the different earthen ponds and analysed according to APHA (2000) for the following parameters [Temperature (Temp.); Dissolved oxygen (DO), pH, total alkalinity (T. alk.); salinity; and ammonia (NH_3)].

A-2-Zooplankton examination

Zooplankton samples were collected by filtering 100 liters of the pond water at each pond through a small standard plankton net (mesh size 45μm) using a plastic container of ten liters capacity according to Santhanam and Srinivasan, (1994).

B-Biochemical examinations

Sampling and analysis of blood

Blood samples from all common carp males were obtained from fish anesthetized with MS222 (50mg/L) and without. Blood samples were collected in heparinized tubes. Tubes were centrifuged at 3000 r.p.m. for 15 minutes and plasma was removed and stored at -20 °C until analysis.

B-1-Acetylcholine esterase

Acetylcholine esterase was measured at 405 nm against air according to Waber (1966) using cholinesterase test kit produced by Diamond Diagnostics, Egypt.

B-2-Protein

Serum total protein content and serum albumin were determined according to King and Wooton (1959).

B-3-Glucose

Glucose determined by glucose oxidase / peroxidase method, was adopted according to Trinder (1969) method using glucose kit, produced by Diamond Diagnostics, Egypt.

B-4-Cortisol

Cortisol levels in plasma were measured by immunological method (Sibar, Perugia, Italy) (Arakawa *et al.*, 1979).

C-Milt properties

Administration of hormones

Common carp were anaesthetized with 0.02% MS-222 and each hormone was injected intraperitoneally. Fish used in control experiments were injected with the same dosage of bovine serum albumin (BSA; Sigma) in phosphate buffered saline. BSA was used as a control for this experiment as it does not elicit immune response (Barton and Iwama 1991).

C-1- Semen collection

Sperm was collected in spring and autumn. The semen was collected by gently pressing the abdomen 14 hrs after intramuscular injection of acetone-dried

common carp pituitaries dissolved in FPS at a dose of 3 mg/kg body weight according to Mairian *et al.*, (1993).

C-2-Spermatozoa cell count and evaluation of motility

Cell counts were carried out as described by Linhart (1991), Counting and evaluation of motility were done by observing the screen monitor. About 20 µl of Spz were placed on a glass microscope slide and ~200 µl of water were added. Motility was evaluated as described by Linhart (1991). Spermatozoa (spz) concentration was measured in milt diluted 4000 times in the immobilizing solution. One drop of diluted milt was placed on a Thomas glass cell and the number of spermatozoa was counted on 8 out of 16 squares of the cell. Milt concentration (10^9 spz ml^{-1}) was calculated as follows: C = counted spermatozoa X dilution of milt (4000) X constant (31,250). Counts were made in quadruplicate and the mean value was recorded.

C-3- Motility testing

The motile fraction of the activated sperm cells viability and the duration of flagellar motion were determined by light microscopy. The semen was diluted 1:100 in SPS prior to microscopic motility testing. The pre-diluted sperm cells were activated by adding HSS in a 30-fold volume. The duration of fast movement was measured (Billard *et al.,* 1995).

Statistical analysis

Data are expressed as Mean± Standard deviation. Data water quality, zooplankton, semen characteristics (count, milt volume, mass motility) and biochemical parameters (Ache. E. cortisol hormone, sugar, protein, and albumin) were subjected to one-way analysis of variance, to determine significant differences between treated and non treated male common carp groups according to SAS (1999). The level of significant difference was set at $P<0.05$.

RESULTS

Our results in Table (1) showed that the highest variation was represented in water temperature followed by ammonia whereas dissolved oxygen, pH, salinity and total alkalinity were slightly changed during different months measured. Water

temperature was highest in Jul. and Aug. 2006 (32.3±0.5 and 33.3±0.6 °C) and declined to 14.2±0.3 °C in Feb.2007. Ammonia ranged from 0.70±0.03 mg/l in Jul 2006 to 0.27±0.03 mg/l in April. 2007.

Table (2) shows that; the highest number of crustacean orders was rotifer followed by copepoda and cladocera. Rotifer was found in highest numbers in Aug and Sept. 2006 (405±9.9and 420±9.7 organism/l) whereas the lowest numbers of rotifer were found in Feb. and March 2007 (98±4.2 and 137±5.2 organism/l). Order copepoda was found in highest numbers in Aug and Sept. 2006 (240±3.5 and 200±3.1 organism/l) while the lowest numbers was observed in Feb. and March 2007 (52±1.2 and 77±2.1 organism/l). Order cladocera also showed that the highest numbers recorded in Aug and Sept. 2006 Aug and Sept. 2006 (148±4.1 and 145±2.2 organism/l) while the lowest numbers was observed in Feb. and March 2007 (20±1.1 and 35±2.1 organism/l).

During spring season the elevation of cortisol, sugar and Ach. E. levels as a consequence of adaptation to stress (reproduction without Anaesthesia. The highest level of cortisol was recorded in treatment 1 (115.2±5.3 ng/ml) where as the lowest level was recorded in control group before Anaesthesia (88.2 ±4.2 ng/ml); the highest value of sugar and Ach. E. was reworded in treatment 3 (120.1±9.2mg/dl) and in treatment 2 (179.4±3.72 µ/mg) before use of MS222 while after use MS222 the lowest values of cortisol, sugar and Ach. E. was observed in treatment 3 (68.2±2.4 ng/ml), treatment 1 (75.3±2.5mg/dl) and treatment 3 (64.2±3.91 µ/mg) respectively as showed in Table 3.

In autumn season the same previous parameters (cortisol, sugar and Ach. E.) were measured and tabulated in table (4). The results showed that there were an increase significant difference in means of cortisol, sugar and Ach. E. before and after using of MS222 in all treatments. The highest values of cortisol (103.6±4.2ng/dl), sugar (93.1±6.8 mg/dl) and Ach. E. (161.2±5.33 µ/mg) was recorded in treatment 2. 3 and 2 before use MS222 respectively. After use MS222,

the lowest means of cortisol (52.3±2.5 ng/ml), sugar (75.3±3.21 mg/dl) and Ach. E (45.9±1.75 µ/mg) was recorded in control group.

The results in table (3 and 4) showed that there were no significant difference in means of serum total protein, albumin and globulin before and after use of MS222 in the same season (spring and autumn).

Table 5 & 6 showed that during spring 2007 sperm count, milt volume and mass motility were found to rise from (1.844±0.040 $x10^9$/ml, 2.2±0.1 ml, and 54.21±2.03% respectively) in control group after use of MS222 to (2.684±0.054 $x10^9$/ml, 7.2±0.2 ml and 87.42±2.33 % respectively) in treatment 1 after use of Anaesthesia. During autumn 2006 sperm count, milt volume and mass motility showed a significant increase between treatments use MS222 and treatments don't use MS222 and this trend was maintained up to all treatments in compared with control group. Sperm count was recorded (1.770±0.054; 1.424±0.026; 1.642±0.022 and 1.128±0.020 $x10^9$/ml respectively) in treatments 1, 2, 3 and control group before Anaesthesia while, sperm count was recorded (1.882±0.046, 1.542±0.026, 1.764±0.026 and 1.172±0.032 $x10^9$/ml respectively) in treatment 1, 2, 3 and control group after Anaesthesia in autumn season.

Milt volume and mass motility was significant in all treatments during their examination in spring season, table (5) showed that the milt volume and mass motility was recorded in treat 1 (6.3±0.3 ml and 75.24±2.41%), treat 2 (3.9±0.2 ml and 73.22±2.6%), treat 3 (5.4±0.3 ml and 69.31±2.35%) and control group (2.2±0.1 ml and 54.21±1.03%) before anaesthesia and increased after anaesthesia up to (7.2±0.2 ml and 87.42±2.33%), (4.7±0.1 ml and 82.44±2.81%), (6.1±0.2 ml and 78.22±3.22%), and (2.9±0.2 ml and 60.22±1.45%) in treatments 1, 2, 3 and control group respectively.

During milt examination in autumn season as regarded in Table 6 observed that milt volume and mass motility were significant increase in male's anaesthesia in compared without anaesthesia in all treatments and control groups. The highest value for milt volume and mass motility % were recorded in treat 1 (3.8±0.2 ml and 76.26±3.22%) after anaesthesia whereas before anaesthesia the lowest value

was recorded in control group (0.5±0.01 ml and 48.31±1.62%) for milt volume and mass motility respectively.

DISCUSSION

In the present study, during summer and winter 2006 the measured environmental factors explained the total variance of crustacean species distribution, suggesting that many other undetermined factors within ponds are also of great importance in structuring the crustacean community; these undetermined factors might include bacteria and planktivorous fishes. These observations were agreement with Havens *et al.* (2000) they found that temperature played a role in determining the dominance of daphnids. The major crustacean zooplankton groups may response differentially to trophic indicators (Pinto-Coelho *et al.*, 2005). Total phosphorus (TP) which suggested being a powerful predictor (Pinto-Coelho, *et al.,* 2005). Monitoring of the live feed density is important in first feeding as well as in the production of live feed, because the feed density has a significant effect on the growth and survival of the fish (Lubzens *et al.*, 1989). Manual counting of rotifer densities is time consuming, and for this reason and others, the production of live food amounts to a significant part of the production costs for marine fish species Morten *et al.*, (2007). Abundance and intensity of copepod showed seasonal changes Sandra *et al.*, 2002. These results differ from information indicating a significant increase in abundance and intensity of copepods during spring and summer (Gonza´lez and Carvajal, 1999). This is due to a reduction in the time lapse between generations (Gonza´lez and Carvajal, 1999) and to greater fecundity and egg viability caused by increased temperature during spring and summer. Among the zooplankton, copepods numerically and by biomass dominate the zooplankton community (Kibirige and Perissinotto, 2003b). The reduced zooplankton biomass observed during winter which was attributed to the lower water temperatures (Hansson, 1997).

The elevation of cortisol and sugar levels as a consequence of adaptation to stress (fish without anaesthesia) and depletion (fish with anaesthesia) and Ach. E. is generally accepted as the main initial factor in the cascade of events that lead to disruption of processes like growth, immune capacity and reproduction. These results are agreement with in fish; the response to stress has many similarities to that of higher vertebrates, as it leads to an activation of the hypothalamic-pituitary-interrenal (HPI) axis, the equivalent of the mammalian hypothalamic-pituitary-adrenal (HPA) axis. In teleost fish, cortisol is the main glucocorticoid produced by the interrenals under stress adaptation. Cortisol has frequently been indicated as a major factor mediating the suppressive effect of stress on reproduction. Previous work showed that prolonged cortisol treatment inhibits pubertal development in male common carp (Consten *et al.*, 2001).

Seasonal changes were changed the cortisol and sugar levels in our results that may be change the seminal properties so; the testicular development in juvenile common carp was inhibited by chronic stress, induced by repeated changes in water temperature. Physiological adaptation to this stressor was accompanied by the elevation of cortisol levels these observations are agreement with Tanck *et al.*, (2000). Also, Consten *et al.*, (2001) they demonstrated that prolonged elevation of plasma cortisol levels in male juvenile common carp, indeed resulted in a retardation of testicular development and a decrease of 11-oxygenated androgen plasma levels, assumed to be involved in the induction of spermatogenesis. Serum total protein and albumin as showed an autumn and spring before and after anaesthesia; there are no significant difference in between treatments.

Our results showed the decline of acetylcholine esterase after using methanesulfonate (MS-222) this observation can be explained by the following. Efficient cholinergic transmission requires accurate targeting of vesicular acetylcholine transporter (VAChT) to synaptic vesicles (SVs). However, the signals that regulate this vesicular targeting are not well characterized (Colgan *et al.* 2007). Markin and Mayer (1985) produced a list of characteristics of an ideal

anaesthetic. In addition to these characteristics, a particular anaesthetic should have a stress-reducing capacity, which ought to block the hypothalamus–pituitary–interrenal (HPI) axis and render the fish unable to respond to additional stressors (Keene *et al.*, 1998). So far, various compounds, including heavy alcohols, ether, chloretone, brominated alcohols, barbiturates, chloral hydrate, urethan, quinaldine, tricaine methanesulfonate (MS-222), chlorbutanol and benzocaine, have been used to anaesthetise fish (Gilderhus and Marking, 1987). As unwanted systemic side effects and limited safety margins have been demonstrated, the use of several of these different compounds has been limited or rejected.

Spring season in our hatchery of Central Laboratory of Aquaculture Research (CLAR) Abbassa abou Hamad Sharkia Egypt is considered the more suitable time for hatchery process so called spawning season, so; in this study we are trying to find another suitable time for reproduction process in male common carp. We selected the autumn season to study the more suitable environmental conditions for semen production (sperm count /ml kg; milt volume ml and mass motility %). We observed that semen production was affected by seasonal changes and these results agreement with many authors; Sexual maturity depends on several factors. It is delayed in cold climates, while it is accelerated in warmer factors environments. This aspect is very well exemplified in the common carp and Chinese carps. Seasonal changes in sperm characteristics have been used to suggest potential impacts of sperm age on viability. Sperm concentration has been shown to increase throughout the spawning season for common carp *Cyprinus carpio* L. (Zhukinskiy and Alekseenko, 1983).

Semen quality was affected by seasonal changes in addition to hormonal stimulation by carp pituitary extract, human chronic gonadotropin and their mixture; so the milt volume and mass motility were increased by hormonal stimulation and using MS222 as anaesthesia, whereas sperm count was not affected these results are agreements with many authors. The increase in sperm density late in the spawning season is probably a result of reduced dilution of semen rather than an increase in sperm production (Shangguan and Crim, 1999).

Since spermatogenesis is highly synchronous and usually completed long before spawning commences (Rideout and Burton, 2000), the production of additional sperm during spawning does not appear possible. Treating males of various aquaculture species with reproductive hormones late in the spawning season serves to increase the dilution of sperm, not to increase sperm production (Shangguan and Crim, 1999), also they added hormone injections have been shown to have no effect on sperm quality in other species which may result in poor quality sperm no longer being identifiable via spermatocrit. Although spermatozoa size was not measured, Rakitin *et al.* (1999) reported no decrease in spermatozoa size throughout the spawning season for Atlantic cod and therefore changes in spermatocrit were attributed to changes in sperm number. Billard *et al.*, (1995) recorded the milt volume in common carp to be 3-6 ml/kg body weight also, fish sperm motility have indicated clearly that fish spermatozoa are quiescent in solutions or buffers isotonic to seminal plasma and show motility when diluted with hypotonic solution in the case of freshwater fishes.

Cacot *et al.*, (2003) reported that in order to improve the technology of milt production and management in *P. bocourti*. Two hormonal treatments were tested to augment spermiation. Treatments involved a single injection of hCG (2000 IU kg^{-1}) or GnRHa (30 mg kg^{-1}) in association with domperidone (3 mg kg^{-1}). The hCG treatment results in the spermiation of most of the males over several weeks (Asturiano *et al.,* 2004). Kusuda, *et al.* (2005) recorded that Spermatozoa were considered motile when the sperm head showed forward movement under the consecutive video frames from 10 s after thawing. Percent motility was determined by assessing the motility of at least 50 randomly selected spermatozoa for each measurement.

CONCLUSION

The reproductive efficacy and some biochemical parameters (cortisol, Ach. E. and sugar) of male common carp are depending on seasonal changes of water

quality and zooplankton community in pre-spawning period which precedes the time of spawning. Finally the water temperature and crustacean community sure that spring season is better than autumn season but we can hatchery common carp in autumn season.

Table 1. Some physico-chemical parameters of water in different months during preparation of common carp males for reproduction in Abbassa earthen ponds.

Month / Item	Temp. °C	pH	Dissolved oxygen (mg/l)	Salinity (g/l)	NH_3 (mg/l)	Total alkalinity (mg/l)
Jul. 06	32.3±0.5a	9.11±0.4a	4.21±0.2c	0.40±0.02a	0.70±0.03a	168±11a
Aug. 06	33.3±0.6a	8.92±0.2a	4.44±0.3c	0.39±0.03a	0.65±0.02a	152±14a
Sept. 06	28.9±0.4b	8.72±0.3a	5.25±0.3b	0.40±0.02a	0.66±0.03a	142±12
Feb. 07	14.2±0.3e	8.12±0.3b	6.42±0.4a	0.32±0.02b	0.41±0.02b	140±16b
March.07	17.2±0.5d	7.75±0.2b	6.22±0.4a	0.37±0.03a	0.32±0.03c	139±9b
April. 07	21.3±0.4c	7.42±0.3c	6.45±0.3a	0.35±0.03a	0.27±0.03c	149±13a

The means with the different liter in the same column for the same item are significant ($P<0.05$)

Table 2. The total number (organism/l) of different zooplankton orders counted in different months during preparation of common carp males for reproduction in Abbassa earthen ponds

Month / Item	Rotifera	Cladocera	Copepoda
Jul. 06	310±8.7a	125±2.3b	107±2.4b
Aug. 06	405±9.9a	148±4.1c	240±3.5b
Sept. 06	420±9.7a	145±2.2c	200±3.1b
Feb. 07	98±4.2a	20±1.1c	52±1.2b
March.07	137±5.2a	35±2.1c	77±2.1b
April. 07	176±6.3a	115±3.2b	90±2.2c

The means with the different liter in the same row for the same month are significant ($P<0.05$)

Table 3. The effect of MS-222 anesthesia and pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some blood biochemical properties of mature male common carp during spring season before and after anesthesia.

Items	T1		T2		T3		T4	
Treat	Before	After	Before	After	Before	After	Before	After
Cortisol ng/ml	88.2±4.2a	74.3±2.6b	115.2±5.3a	78.3±3.1b	102.4±5.2a	72.3±2.7b	97.4±3.3a	68.2±2.4b
Sugar mg/dl	98.25±7.1a	88.3±4.2b	103.3±5.3a	75.3±2.5b	109.2±8.7a	88.3±3.1b	120.1±9.2a	98.2±3.1b
Ach. E. µ/mg	159.2±4.66a	67.8±2.11b	172.3±3.54a	73.4±3.14b	179.4±3.72a	66.3±3.26b	177.3±4.4a	64.2±3.9b
T. protein g/dl	5.85±0.2a	5.82±0.22a	5.86±0.2a	5.88±0.19a	5.92±0.23a	5.87±0.17a	5.58±0.19a	5.61±0.18a
S. albumin g/dl	1.54±0.16a	1.55±0.15a	1.57±0.15a	1.55±0.14a	1.62±0.13a	1.59±0.15a	1.55±0.13a	1.59±0.16a
globulin g/dl	4.31±0.22a	4.25±0.21a	4.29±0.21a	4.35±0.35a	4.30±0.19a	4.29±0.32a	4.03±0.17a	4.03±0.42a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

Table 4.The effect of MS-222 anesthesia and pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some blood biochemical properties of mature male common carp during autumn season before and after anesthesia.

Items	T1		T2		T3		T4	
Treat	Before	After	Before	After	Before	After	Before	After
Cortisol ng/ml	78.6±3.3a	52.3±2.5b	96.3±4.3b	73.6±2.7b	103.6±4.2a	81.6±3.15b	89.4±3.6a	71.3±3.22b
Sugar mg/dl	86.2±5.1a	75.3±3.21b	91.3±4.7a	78.8±3.3b	89.7±4.7a	81.1±2.7b	93.1±6.8b	85.6±3.7a
Ach. E. µ/mg	133.2±4.22a	45.9±1.75b	152.3±5.22a	63.4±2.24b	161.2±5.33a	54.3±3.11b	158.4±4.33a	52.3±2.11b
T. protein g/dl	5.32±0.17b	5.22±0.19a	5.26±0.22b	5.17±0.18a	5.82±0.19a	5.79±0.2a1	5.46±0.18a	5.43±0.17a
S. albumin g/dl	1.43±0.15a	1.41±0.13a	1.49±0.14a	1.48±0.13a	1.59±0.15a	1.61±0.16a	1.49±0.15a	1.50±0.14a
globulin g/dl	3.89±0.19b	3.83±0.25a	3.77±0.18b	3.69±.29a	4.23±0.18a	4.19±0.44a	3.97±0.16a	3.94±0.36a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

Table 5. Effect of pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some properties of semen before and after MS 222 anesthesia of male common carp during spring season.

	T1		T2		T3		T4	
Items Treat	Before	After	Before	After	Before	After	Before	After
Body weight g	1480±36.2a	1470±43.6a	1520±35.3a	1490±42.5a	1490±35.7a	1510±33.9a	1470±28.7a	1500±30.5a
Body length cm	45.2±2.88a	46.2±2.58a	48±1.6a	47±1.3a	46±1.5a	47±2.1a	48±1.6a	47±1.8a
Sperm count $X10^9$/cmm	1.844±0.040a	1.865±0.051a	2.660±0.075ba	2.684±0.054ba	2.324±0.029a	2.351±0.038a	2.520±0.027a	2.561±0.036a
Milt volume/ml	2.2±0.1b	2.9±0.2a	6.3±0.3b	7.2±0.2a	3.9±0.2b	4.7±0.1a	5.4±0.3b	6.1±0.2a
Mass motility %	54.21±1.03b	60.22±1.45a	75.24±2.41b	87.42±2.33a	73.22±2.6b	82.44±2.81a	69.31±2.35b	78.22±3.22a
Time anaesethia min.		5.50±0.17a		5.15±0.18a		5.34±0.23a		5.45±0.22a
Recovery time min.		2.15±0.12a		2.19±0.10a		2.32±0.24a		2.21±0.19a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

quality and zooplankton community in pre-spawning period which precedes the time of spawning. Finally the water temperature and crustacean community sure that spring season is better than autumn season but we can hatchery common carp in autumn season.

Table 1. Some physico-chemical parameters of water in different months during preparation of common carp males for reproduction in Abbassa earthen ponds.

Month / Item	Temp. °C	pH	Dissolved oxygen (mg/l)	Salinity (g/l)	NH_3 (mg/l)	Total alkalinity (mg/l)
Jul. 06	32.3±0.5a	9.11±0.4a	4.21±0.2c	0.40±0.02a	0.70±0.03a	168±11a
Aug. 06	33.3±0.6a	8.92±0.2a	4.44±0.3c	0.39±0.03a	0.65±0.02a	152±14a
Sept. 06	28.9±0.4b	8.72±0.3a	5.25±0.3b	0.40±0.02a	0.66±0.03a	142±12
Feb. 07	14.2±0.3e	8.12±0.3b	6.42±0.4a	0.32±0.02b	0.41±0.02b	140±16b
March.07	17.2±0.5d	7.75±0.2b	6.22±0.4a	0.37±0.03a	0.32±0.03c	139±9b
April. 07	21.3±0.4c	7.42±0.3c	6.45±0.3a	0.35±0.03a	0.27±0.03c	149±13a

The means with the different liter in the same column for the same item are significant ($P<0.05$)

Table 2. The total number (organism/l) of different zooplankton orders counted in different months during preparation of common carp males for reproduction in Abbassa earthen ponds

Month / Item	Rotifera	Cladocera	Copepoda
Jul. 06	310±8.7a	125±2.3b	107±2.4b
Aug. 06	405±9.9a	148±4.1c	240±3.5b
Sept. 06	420±9.7a	145±2.2c	200±3.1b
Feb. 07	98±4.2a	20±1.1c	52±1.2b
March.07	137±5.2a	35±2.1c	77±2.1b
April. 07	176±6.3a	115±3.2b	90±2.2c

The means with the different liter in the same row for the same month are significant ($P<0.05$)

Table 3. The effect of MS-222 anesthesia and pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some blood biochemical properties of mature male common carp during spring season before and after anesthesia.

Items	T1		T2		T3		T4	
Treat	Before	After	Before	After	Before	After	Before	After
Cortisol ng/ml	88.2±4.2a	74.3±2.6b	115.2±5.3a	78.3±3.1b	102.4±5.2a	72.3±2.7b	97.4±3.3a	68.2±2.4b
Sugar mg/dl	98.25±7.1a	88.3±4.2b	103.3±5.3a	75.3±2.5b	109.2±8.7a	88.3±3.1b	120.1±9.2a	98.2±3.1b
Ach. E. µ/mg	159.2±4.66a	67.8±2.11b	172.3±3.54a	73.4±3.14b	179.4±3.72a	66.3±3.26b	177.3±4.35a	64.2±3.9b
T. protein g/dl	5.85±0.2a	5.82±0.22a	5.86±0.2a	5.88±0.19a	5.92±0.23a	5.87±0.17a	5.58±0.19a	5.61±0.18a
S. albumin g/dl	1.54±0.16a	1.55±0.15a	1.57±0.15a	1.55±0.14a	1.62±0.13a	1.59±0.15a	1.55±0.13a	1.59±0.16a
globulin g/dl	4.31±0.22a	4.25±0.21a	4.29±0.21a	4.35±0.35a	4.30±0.19a	4.29±0.32a	4.03±0.17a	4.03±0.42a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

Table 4.The effect of MS-222 anesthesia and pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some blood biochemical properties of mature male common carp during autumn season before and after anesthesia.

Items / Treat	T1		T2		T3		T4	
	Before	After	Before	After	Before	After	Before	After
Cortisol ng/ml	78.6±3.3a	52.3±2.5b	96.3±4.3b	73.6±2.7b	103.6±4.2a	81.6±3.15b	89.4±3.6a	71.3±3.22b
Sugar mg/dl	86.2±5.1a	75.3±3.21b	91.3±4.7a	78.8±3.3b	89.7±4.7a	81.1±2.7b	93.1±6.8b	85.6±3.7a
Ach. E. µ/mg	133.2±4.22a	45.9±1.75b	152.3±5.22a	63.4±2.24b	161.2±5.33a	54.3±3.11b	158.4±4.33a	52.3±2.11b
T. protein g/dl	5.32±0.17b	5.22±0.19a	5.26±0.22b	5.17±0.18a	5.82±0.19a	5.79±0.2a1	5.46±0.18a	5.43±0.17a
S. albumin g/dl	1.43±0.15a	1.41±0.13a	1.49±0.14a	1.48±0.13a	1.59±0.15a	1.61±0.16a	1.49±0.15a	1.50±0.14a
globulin g/dl	3.89±0.19b	3.83±0.25a	3.77±0.18b	3.69±.29a	4.23±0.18a	4.19±0.44a	3.97±0.16a	3.94±0.36a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

Table 5. The effect of pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some properties of semen before and after MS 222 anesthesia of male common carp during spring season.

Items / Treat	T1		T2		T3		T4	
	Before	After	Before	After	Before	After	Before	After
Body weight g	1480±36.2a	1470±43.6a	1520±35.3a	1490±42.5a	1490±35.7a	1510±33.9a	1470±28.7a	1500±30.5a
Body length cm	45.2±2.88a	46.2±2.58a	48±1.6a	47±1.3a	46±1.5a	47±2.1a	48±1.6a	47±1.8a
Sperm count $X10^9$/cmm	1.844±0.040a	1.865±0.051a	2.660±0.075ba	2.684±0.054ba	2.324±0.029a	2.351±0.038a	2.520±0.027a	2.561±0.036a
Milt volume/ml	2.2±0.1b	2.9±0.2a	6.3±0.3b	7.2±0.2a	3.9±0.2b	4.7±0.1a	5.4±0.3b	6.1±0.2a
Mass motility %	54.21±1.03b	60.22±1.45a	75.24±2.41b	87.42±2.33a	73.22±2.6b	82.44±2.81a	69.31±2.35b	78.22±3.22a
Time anaesethia min.		5.50±0.17a		5.15±0.18a		5.34±0.23a		5.45±0.22a
Recovery time min.		2.15±0.12a		2.19±0.10a		2.32±0.24a		2.21±0.19a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

Table 6. The effect of pituitary extract (T1), HCG hormone (T2) and their mixture (T3) on some properties of semen before and after MS 222 anesthesia of male common carp during autumn season.

Items / Treat	T1		T2		T3		T4	
	Before	After	Before	After	Before	After	Before	After
Body weight g	1450±31.2a	1440±33.2a	1540±30.2a	1470±55.5a	1560±39.2a	1530±43.2a	1490±28.7a	1520±42.3a
Body length cm	46.3±2.65a	44.2±2.45a	47±1.5a	48±1.9a	47±1.8a	48±1.5a	47±1.8a	48±1.4a
Sperm count $X10^9$/cmm	1.128±0.020a	1.172±0.032a	1.770±0.054a	1.882±0.046a	1.424±0.026b	1.542±0.026a	1.642±0.022b	1.764±0.026a
Milt volume ml	0.5±0.01b	0.9±0.1a	2.6±0.1b	3.8±0.2a	2.1±0.1b	2.7±0.1a	2.2±0.1b	2.9±0.2a
Mass motility %	48.31±1.62a	51.11±2.45a	64.42±3.21b	76.26±3.22a	62.24±2.46b	69.42±2.84a	64.22±2.22b	73.42±2.32a
Time anaesethia min.		5.1±0.40a		4.55±0.20a		5.15±0.21a		5.20±0.17a
Recovery time min.		1.53±0.18a		2.15±0.16a		2.25±0.22a		2.13±0.18a

The means with the same liter in the same row for the same item in the same group are not significant (P>0.05)

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تاثير التغيرات الموسمية والحقن الهرمونى على بعض التغيرات البيوكيميائية وكفاءة السائل المنوى فى ذكور المبروك العادى

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تم استخدام ١٤٤ ذكر من المبروك العادى بمتوسط وزن ١٥٠٠جرام جمعت من مزرعة المعمل المركزى لبحوث الثروة السمكية بالعباسة ابو حماد شرقية مصر. وقسمت الى اربع مجموعات الاولى مجموعة السيطرة والثانية معاملة الاولى وتم حقنها بمستخلص الغدة والثالثة المعاملة الثانية وتم حقن الذكور بهرمون الجنادوتربين والرابعة المعاملة الثالثة وتم حقن ذكورها بخليط من الغدة والهرمون. وتم تجميع السائل المنوى فىفصل الخريف ٢٠٠٦ والربيع لعام ٢٠٠٧. وتم ملاحظة فرق بين الخريف والربيع. وقبل تجميع السائل المنوى وعينات الدم تم تخدير الاسماك بمخدر MS222 وتوجد علاقة معنوية بين العوامل البيوكيميائية قبل وبعد التخدير حيث توجد زيادة معنوية فى مستوى الاستيل كولين استراز والكورتيزول والسكر قبل التخدير عنها بعد التخدير داخل الفصل الواحد بينما معدل البروتين والالبومين يظل دون تغير. تم ضبط جودة المياة للتفريخ قى الربيع والخريف من حرارة وملوحة واكسجين وامونيا. وتم فحص الهائمات الحيوانية المختلفة فى فصلى الصيف والستاء.