Effects of three different sources of pituitary extract on gonadal inducer in male and female pacu (*Piaractus mesopotamicus)*

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ABSTRACT. Male and female pacus (*Piaractus mesopotamicus*) were induced with broiler chicken (BCPE), rabbit (RPE), and carp (CPE) pituitary extracts. The parameters for spermatic vigor, progressive motility, fertilization and hatching rate, according to semen origin, did not show any differences among the treatments (P>0.05). RPE produced the lowest (P<0.05) semen volume and the highest spermatozoa number and concentration (P<0.05). No difference was observed (P>0.05) in the total rate of abnormal spermatozoa among the treatments, but the secondary pathologies were higher (P<0.05) in the treatment with RPE. The females induced with RPE did not present stimulus for hatching. No differences (P>0.05) between BCPE and CPE were found for accumulated thermal unit, number of oocytes/spawning grams, fertilization and hatchery rates according to treatment origin. The result indicates that BCPE use is recommended for gonadal induction of *P. mesopotamicus*.

Key words: aquaculture, fish, hatching, semen, spermatozoa, oocytes.

RESUMO. Efeito de três diferentes fontes de extrato de pituitárias na indução gonadal em machos e fêmeas de pacu (*Piaractus mesopotamicus*). Machos e fêmeas de (*Piaractus mesopotamicus*) foram induzidas com extrato de hipófise de frango de corte (BCPE), coelho (RPE) e de carpa (CPE). Os parâmetros vigor espermático, motilidade progressiva, taxa de fertilização e eclosão de acordo com a origem do sêmen, não apresentou diferença (p>0,05) entre os tratamentos. O tratamento com BCPE produziu o menor volume de sêmen (p<0,05), o número e a concentração de espermatozóides foram os mais altos (p<0,05). Não foi observado diferença (p>0,05) para o taxa de espermatozóides com anormalidades nos tratamentos, mas as patologias secundárias foram elevadas (p<0,05) no tratamento com RPE. As fêmeas induzidas com RPE não apresentaram estímulo para a desova. Não houve diferença (p<0,05) entre BCPE e CPE para a unidade térmica acumulada, número de ovócitos liberados/g de desova, taxa de fertilização e desova de acordo com a origem dos tratamentos. O resultado indica que o uso de BCPE é recomendado para indução gonadal do *P. mesopotamicus*.

Palavras chave: aqüicultura, peixe, desova, sêmen, espermatozóides, ovócitos.

Introduction

The search for native fish with good animal science potential has resulted in *pacu* (*Piaractus mesopotamicus*) being one of the most utilized species for research in Brazil. Among its animal science characteristics, the high capacity of survival in captivity may be quoted (Castagnolli, 1992).

The *P. mesopotamicus* is a migratory species in fresh water and, when in confined environment, it depends on hormonal induction to reproduce successfully (Castagnolli, 1992). The first studies on pacu reproduction were carried out in the seventies, using human Chorionic Gonadotropin (hCG) as gonadal inducer (Godinho *et al.*, 1977). During the eighties, partially purified gonadotropin and salmon pituitary extract were used by Castagnolli and Donaldson (1981) in pacu reproduction (*Piaractus mesopotamicus*). Romagosa *et al.* (1985) induced the *Piaractus mesopotamicus* with crude salmon pituitary extract

and hCG, and Godinho and Godinho (1986) used carp pituitary extract. Many advantages justify the widespread use of pituitary method, when compared to other hormonal induction methods. According to Donaldson and Hunter (1983), the pituitary method is simple and practical. Avian pituitary extract has also been tested as a possible fish gonadal inducer (Amaral Jr., 1995; Yu *et al.*, 1995; Barroso, 1999).

Therefore, the purpose of this research was to study the efficiency of broiler chicken and crude rabbit pituitary extract in the reproduction of male and female pacu (*P. mesopotamicus*), compared to carp crude pituitary extract, in order to find a new, low-cost alternative to traditional carp pituitary extract.

Material and methods

Two experiments were carried out on the reproduction of pacu (*P. mesopotamicus*), from December 2001 to January 2002, in the Fishery Station of Universidade Estadual de Maringá/Codapar, district of Floriano, Maringá, State of Paraná, southern Brazil.

Seventy-eight *P. mesopotamicus* were selected (42 males and 36 females) and stocked into a 600 m^2-tank, with 0.5 Kg/m^2 density. During six months, these animals received extruded ration (*ad libtum*), extruded feed with 38% protein, 7% crude fiber, 4% ether extract, 13% dry matter, 4.5% calcium, 0.8% phosphorus, and 12% wet.

Males and females with apparent potential for induction were selected. The males released semen when their abdomen was pressed. The females presented a convex belly, soft abdomen and red urogenital orifice.

Fish were marked in the laboratory on the back fin with telephone wire and divided into groups, according to sex. Each tank had a fifty-centimeter-water column in constant water flow, water temperature at 25.5±1.5ºC, and 4 to 7 mg/L of dissolved oxygen kept by the water flow or through compressed air from an automatic compressor.

Pituitary collection

Broiler chicken pituitaries were obtained from 45-days animals (mean age) from a slaughterhouse in Maringá. Rabbit pituitaries were collected from animals slaughtered at the Experimental Farm of the Universidade Estadual de Maringá (UEM), in Iguatemi, State of Paraná. The rabbits were 60 to 70 days old, a suitable age for slaughtering this species. Carp pituitary was acquired in the market, ready to be used.

Broiler chicken and rabbit pituitaries were obtained from an initial incision in the occipital region, crossing between the eye and the ear, reaching the center of the mouth. The animal brain and the sella turcica were exposed and the pituitary was extracted. In the birds, the incision was made with a sharp knife; in the rabbits, with a butcher's saw. Odontological curettes or injection needles (25X8) were used to extract pituitary from the sella turcica. After extraction, the rabbit and broiler chicken pituitaries were placed into glass recipients filled with pure acetone, and remained totally immersed for ten hours. The acetone was then changed, and the material was kept for another ten hours. After that, the pituitaries were weighed, placed on a filtering tissue, and taken into a desiccator.

The carp pituitary extract used in the experiment was obtained from a trusted supplier of UEM fish farming station.

Administration of pituitary extracts

Fish were selected and transported to the laboratory, where they received the respective pituitary extracts. The pituitaries were macerated in 100 mL mortars and a physiological solution of 0.7% of NaCl, in a proportion of 4-mg pituitary per 1 mL-saline solution (Woynarovich and Horváth, 1983). The carp pituitary extract dosage utilized was recommended by Woynarovich and Horváth (1983) for species that have completed spawning. For chicken pituitary extract, a fallow-up dosage was recommended by Barroso (1999). When rabbit pituitary extract was used, the dosage was based on pilot work and late experiments with carp. The procedure of induction was repeated four times for females and five times for males, using the schedule indicated in Table 1.

Table 1. Schedule of application of bird, rabbit and carp pituitary extracts (mg/Kg of living weight) in *P. mesopotamicus*.

SEX	DOSE	PITUITARY EXTRACT		
		CARP	BROILER CHICKEN	RABBIT
MALE (42)[1]	SINGLE	3.0	5.0	7.0
FEMALE (36)[1]	1st	0.5	1.0	1.4
	2nd	5.0	9.0	12.6

[1]Number of animals used into parenthesis.

One by one, the males were taken out of the manipulation tanks and placed on 30-density foam mattress and heads covered with a wet cotton towel. A 5 mL syringe, with a 27x7 needle, was used to proceed the hormone injection, via intra-peritoneal. The females received two pituitary extract applications with an interval of 12 hours between

each one. Water temperature was constantly observed with a thermometer of Celsius graduation. After reaching 260 of accumulated thermal unit (ATU) (Ceccarelli *et al.,* 2000), the female pacu received light abdominal pressure. The ATU was determined every hour, after the last hormonal application until spawning time (Ceccarelli *et al.*, 2000). When 260 ATU was reached, the males received abdominal pressure to release the sperm. The male fish received a single dose of pituitary extract, four hours before the last hormonal administration in the female, in order to do the spermogram.

Experiment I – Observation of quali-quantitative parameters of male and female gametes

Each animal was taken out of the tanks to have their gametes collected. Following that, the genital region and the anal fin of the fishes were dried with a tissue paper and the extrusion made by light abdominal pressure. Oocytes were collected into a plastic bowl and semen into a glass, properly identified and transferred into syringes of several sizes, in order to measure their volume and proceed with the next analysis. After that, the males and females were taken back to their tanks. From the collected semen of each pacu, the volume, sperm concentration, progressive motility, spermatic vigor, morphopathology, and totality of spermatozoa collection were analyzed (Sorensen Jr., 1979).

The summaries of the procedure are shown below:

Volume: abdominal massage was used until semen liberation occurred, which was measurable in syringes of several sizes.

Concentration of spermatozoa: the semen was diluted in a small beaker, using the Shalli's pipette (0.02 mL) in 40 mL of buffered formal saline (Hancock, 1951), resulting in the dilution of 1:2000. After dilution, the Neubauer counting was carried out in phase contrast microscopic, at 40X.

Progressive motility and spermatic vigor: a semen drop was diluted with eight distilled water drops at room temperature in a slide of optics microscopy. Afterwards, it was taken to the microscope of phase contrast, magnified 40X and analyzed through subjective method, both variants. For the progressive motility variant, a score from 0 to 100% was used and, for the spermatic vigor, a score from 0 to 5 points.

Morphology: for this analysis, two smears were prepared on microscopic slides (Barth and Oko, 1989) by diluting the semen in buffered formal saline in a proportion of 1:2000 (semen/diluted solution, respectively). The smears were stained by Bengal Rose method (Conn, 1918), dried and taken to the microscope of phase contrast magnified 40X. 200-230 spermatozoa were counted among each slide with smears of liberated semen of each animal. Bent tail, coiled tail, crooked tail and small head were considered primary pathology. Shoehook, headless, tailless and proximal cytoplasmatic drop were considered secondary pathology.

With the values of individual seminal volume, sperm concentration and the total spermatozoa number/semen release was calculated.

After the extrusion of each female, the oocytes were weighed in scales of 400 g, with sensibility of two shells, in order to withdraw three one-gram samples and verify oocytes productivity (quantity of oocytes/g of spawning).

The experimental design was completely random, with three treatments arranged in factorial of three hormones and four repetitions (weeks) for female and five repetitions (weeks) for male per treatment. Each animal was considered an experimental unit.

The semen volume, sperm concentration, total number of sperm, progressive motility, spermatic vigor, spermatic morphopathology, spawning rate, average number of spawning oocytes/g, and ATU were analyzed separately, according to the statistic model below:

$$Yijk = \mu + Hi + Sj + HSij + \varepsilon ijk$$

Yijk = observation in relation to male or female (k), receiving the hormone (i) in the week (j);

μ = general constant;

Hi = hormone effect;

Sj = week effect;

HSij = hormone interaction (i) and the week (j);

εijk = random mistake associated to the observation of animal (k) received hormone (i) in the week (j);

Experiment II – Evaluation of reproductive performance

Fertilization and hatching rates, referring to pituitary extract used in both sexes were evaluated in experiment II.

After extrusion of oocytes or semen of the animal treated according to pituitary extract doses, as indicated in Table 1, fertilizations were performed. The spawn was collected in clean, dry and identified plastic bowls. After that, the semen was put over the spawning and a goose feather carefully homogenized the ova. A new feather was used for each spawning. Every spawning (females induced with carp, chicken

and rabbit pituitary extract) was divided in three equal oocyte portions and fertilized with semen of males induced with carp, chicken and rabbit pituitary extract. This resulted in nine treatments (oocyte combination X semen). The ova were hydrated for about one minute after receiving the semen and put into 60-liter incubators, in constantly renewed water, at 5 l/min.

After nine hours of incubation, the fertilization index was recorded, at gastrula stage (25,5±,5C°). The hatching rate ranged from 16 to 18 hours after beginning the incubation at room temperature. In order to calculate the fertilization percentage and the hatching rate, a siphon removed three samples from the incubator and 100 eggs were counted in a 30X stereoscope. Considering the fertilization rate, the embryos that had gastrula formation were considered viable. Concerning the hatching rate, larvae moving inside the eggs minutes before hatching were considered viable. Three sample averages were taken in three scores. The experimental design was completely randomized. The treatments were arranged in a 3X3 factor (three hormones in males and three hormones in females). The experimental unit was the junction of male and female gametes.

Fertilization and hatching variables were analyzed according to statistic model, considering the hormones administered in male and female animals:

$$Yij = \mu + Mi + Fj + MFij + Sk + \varepsilon ij$$

Yijk = observation of hormones (i) in male with hormone (j) in female, (k) in the week;

μ = general constant;

Mi = hormone (i) in male;

Fj = hormone (j) in female;

MFij = male and female hormones interaction;

Sk = week effect (k);

εijk = random mistake associated to the hormone (i) in male with hormone (j) in female, (k) in the week.

The statistic analysis was made using the SAS (1992) program.

The weight variables (male and female), sperm concentration, semen volume, number of total sperm, ATU (female), average number of spawning oocytes/g, fertilization, and hatching rates were analyzed through GLM procedure, applying Tukey test ($P<0.05$). To the progressive motility and spermatic vigor variables, Gamma distribution with function of Log link and GENMOD analysis procedure were applied; to the morphopathological variables, distribution was Gamma with function of power inverse link and GENMOD procedure. At last, x2 (Square Qui) ($P<0.05$) was used in the spawning rate.

Results and discussion

Production and analysis of *P. mesopotamicus* semen

The averages of semen volume, sperm concentration, total spermatozoon number/release semen, progressive motility and spermatic vigor obtained from different gonadal inductors are shown in Table 2. The average weight of the animals used in the treatment did not vary ($P>0.05$); thus, it did not interfere with the parameters.

Table 2. Animal average weight, carp, rabbit and broiler chicken pituitary extracts effects on semen volume, spermatic concentration, total number of spermatozoa number/released semen, progressive motility, spermatic vigor, fertilization and hatching rates according to semen origin in *P. mesopotamicus* male reproducers.

Parameters	Pituitary Extract		
	Broiler chicken	Rabbit	Carp
Animal average weight (kg)	2.55±0.3a	2.65±0.5a	2.42±0.3a
Semen volume (ml)	11.2±2.1a	1.8±0.7b	12.0±2.7a
Spermatozoa concentration (mm^3)[1]	21.1±1,2b	31.0±3,2a	20.5±2,3b
Total spermatozoon number/release semen[2]	23.9±2,1a	1.13±0,5b	25.1±3,1a
Progressive motility (%)	72.8±5.4a	80.0±4.1a	76.8±7.3a
Spermatic vigor (0 to 5 score)	2.71±0.1a	2.78±0.1a	2.71±0.2a
Fertilization according to semen origin (%)	52.7±3.4a	50.2±2.1a	57.2±2.2a
Hatchery according to semen origin (%)	50.9±1.6a	50.0±3.1a	56.6±2.5a

Different letters on the same row ($P<0.05$); [1]number $x10^6$; [2]number $x10^{10}$.

The average of semen volume (11.2 mL) produced by the *P. mesopotamicus* induced with extract of broiler chicken's pituitary (BCPE) did not differ ($P<0.05$) from the ones induced with of carp pituitary extract (CPE) (12 mL). However, the *P. mesopotamicus* induced with rabbit pituitary extract (RPE) resulted in the lowest semen volume (1.8 mL) (Table 2).

The explanation for the reduced semen volume of fishes induced with rabbit extract pituitary could be related to the rabbits' age (60 to 70 days old) when abated. When the pituitaries were collected, they might have contained low FSH and LH rates because of sexual immaturity. Under normal breeding conditions, rabbits can reach puberty in three or four months, which is considered a normal time for animal's liberation of gametes and manifestation of sexual behavior (Hafez and Hafez, 2000). Animal pituitary extract between 60 and 70 days was used because this is the age for commercial

slaughter. On the other hand, the birds were slaughtered before the gonadal prime maturity, but the pituitary responded positively.

The concentration of spermatozoa/mm^3 was lower ($P<0.05$) in animals treated with CPE and BCPE when compared to animals treated with RPE (Table 2). The highest spermatic concentration observed in fish treated with RPE may be related to the lowest volume observed in fish treated with CPE and BCPE. From this perspective, the results observed by Silveira *et al.* (1990) were considered, working with *P. mesopotamicus* induced by hCG, obtaining an average volume of 5.02 mL and sperm concentration of about $28x10^6$/mm^3.

These results are equal to those observed in the animals induced with RPE, where a mean semen volume of 1.8 mL and a mean concentration of $31x10^6$ were obtained. Ginzburg (1972) reported the sperm concentration in mm^3 in many species; he observed means of about $2.51x10^6$ in sturgeon (*Huso huso*), possibly reaching $52x10^6$ in perch (*Perca fluviatilis*), or $73x10^6$ in *Caspialosa kessleri*.

The average semen concentration sperm/mm^3 found in this study for fish treated with BCPE was $21x10^6$. It suggests that induced action in sperm production, according to literature, has good concentration associated with semen volume and may assure efficient oocyte fertilization. The total spermatozoa's number/released semen is directly related to volume and concentration. The total sperm number seems to be lower ($P<0.05$) in animals induced by RPE when compared to the ones induced by BCPE and CPE (Table 2). To assure the fertilization of all oocytes, it is necessary to obtain good production of semen with good concentration, in order to achieve sufficient sperm quantity. In this study with *P. mesopotamicus*, an increase of total cell production was observed in the control (CPE). Equal increase was observed in the animals treated with BCPE, comparing to Bedore (1999), who induced the animals with CPE, and also comparing them with non-induced animals. The author observed that the induced animals produced a total of $26.3x10^{10}$ sperm, but the total production obtained in controls was of $5.7x10^{10}$ sperm, where an increase of the total cell number was observed. The same researcher observed identical results when inducing the piracanjuba (*Brycon orbignyanus)*, where animals treated with CPE produced a total sperm number higher than animals not induced ($14x10^{10}$ and $8.6x10^{10}$, respectively). Pardo-Carrasco (2001) studied the quality of yamú semen (*Brycon siebenthalae)* induced with CPE, GnRH-a (analogue mammalian gonadotropin releasing hormone) and verified that animals treated with CPE produced ten times more semen when compared to the controls, but there was no increase in total sperm number. There was just more fluidity in semen, at lower concentration.

When pacu's (*P. mesopotamicus)* spermatic motility and spermatic vigor were analyzed, there was no difference ($P>0.05$) among the three pituitary extract treatments used. Progressive motility and spermatic vigor do not seem to be affected by the origin of the pituitary extract used, but rather by other factors, such as species, animal's age, sperm concentration and mainly on water physical and chemical characteristics (Ginzburg, 1972). In some species, progressive motility may be affected by the inducer hormone, as verified by Kucharczyk *et al.* (1997), who induced the bream (*Abramis brama*) to reproduction using bream and carp pituitary extract and hCG in two doses. The average volume of semen released was 3.5 to 4.0 mL, with progressive motility of 80%, with the control group average semen volume being 1.6 mL, with a motility of 22.8%. Pituitary extract administration in *P. mesopotamicus* seems to increase semen volume and, therefore, the total sperm number, but it did not affect progressive motility or spermatic vigor.

Fertilization between oocytes and semen under different treatments did not differ ($P>0.05$), with the same result observed in the hatching rate (Table 2). It is important to remark that there was a tendency towards lower averages of fertilization and hatching rates in the ova fertilized with semen from male animals treated with RPE, when compared to the ones treated with CPE and BCPE. Coser *et al.* (1992) evaluated the curimatá-pacu's (*Prochilodus marggravii*) semen capacity of fertilization, using CPE, and obtained values of 96.4%. Lahnsteiner *et al.* (1998) emphasized that, in rainbow trout (*Oncorhynchus mykiss*), good quality semen should show progressive motility over 75%, fertilizing, therefore, 80% of ova. In this study, with *P. mesopotamicus*, the average of progressive sperm motility was observed in all treatments and varied from 70 to 80%, but fertilization did not exceed the average of 57%. The fertilization may have been affected not only by oocytes quality, but by different factors, such as high quantity of abnormal sperm, for example. The motility, volume and vigor of animals treated with BCPE and CPE were the same and probably did not influence fertilization. In mammals, fertilization rate is ordinarily related to the percentage of abnormal sperm, according to Hafez and Hafez (2000), who assert that pathology rates over 20% can reduce the fertilization rate in

female animals.

When the sperm morphology was studied, normality rates of 62% were verified in the male animals treated with BCPE and 63% for CPE (P>0.05), but 50.6% for RPE (P<0.05). When sperm pathologies considered secondary, such as shoehook, tailless and headless, were analyzed, a higher percentage (P<0.05) was observed in *P. mesopotamicus* induced with RPE (35.9%), compared to the ones induced with BCPE (25.9%) and with CPE (24.6%). Regarding to more common primary pathologies, such as bent tail, coiled tail, crooked tail and small head, the results were identical (P>0.05) in the three treatments (Figure 1), with the following percentages: 12.1% for BCPE induction, 12.4% for CPE induction, and 13.5% for RPE.

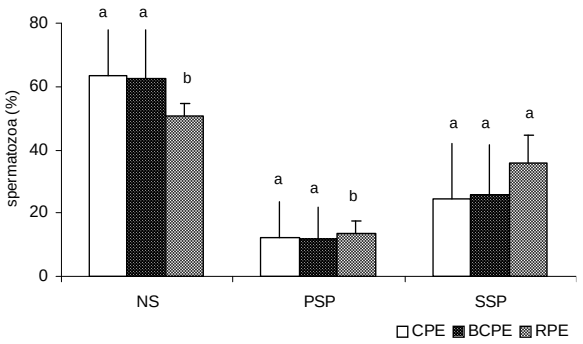

Figure 1. Influence treatments of *P. mesopotamicus* to carp pituitary extract (CPE), broiler chicken pituitary extract (BCPE) and rabbit pituitary extract (RPE) in normal spermatozoa (NS) and primary spermatozoa pathology (PSP) and secondary spermatozoa pathology (SSP).

In this experiment, the secondary and primary pathologies reached an average of 37.5% in animals induced with BCPE and control (CPE) and of 49.4% in the ones induced with RPE. These values can be considered high when compared with mammals. The hypotheses raised for that situation may relate to inadequate diet of reproductive animals, advanced or rather early age, and consanguinity. The data obtained from semen of animals induced with RPE were interesting, the rate of primary spermatic pathologies being similar to that of animals treated with BCPE and CPE. It was not possible, however, to analyze whether the rates of pathologies are related to the inducers, which may cause changes during the spermatozoa formation. This kind of information is important to verify pathologies influence on oocyte fertilization rates, as in the mammal's case (Colégio Brasileiro de Reprodução Animal, 1998), although Nagahama (1983) concluded that there are few differences between mammal and fish spermatogenesis.

Evaluation of *P. mesopotamicus* spawning

The spawning of females with identical average weight (P<0.05), under treatment with CPE, BCPE and RPE was evaluated. Ova weight, oocytes per gram of ova, fertilization rate and hatching rate were evaluated in the CPE and BCPE treatments, but it was not possible to evaluate females treated with RPE, since there was no response from them. Data are in Table 3.

Table 3. Average animal weight and treatment effects with carp, broiler chicken and rabbit hypophysis extract on *P. mesopotamicus* spawning induction, accumulated thermal unit, average number of oocytes/g of spawning.

Parameters	Pituitary Extract		
	Broiler Chicken	Rabbit	Carp
Animals weight average (Kg)	2.7±0.4a	2.6±0.8a	2.9±0.5a
Spawning rate (%)	75.0±20a	0b	83.0±32a
Accumulated Thermal Unit (TAU)	291.0±16a	-	263.0±21a
Average oocytes number/g of spawning	1.497±234a	-	1.461±228 a
Fertilization rate (%)	49.6±9.2a	-	57.0±12a
Hatchery rate (%)	46.6±7.9a	-	57.0±9.9a

Different letters on the same row (P<0.05).

The number of *P. mesopotamicus* that spawned while induced by BCPE was similar to those induced by CPE (P>0.05), but the animals treated with RPE did not react satisfactorily to the spawn (Table 3). This lack of response is probably due to the same hypothetical reasons already mentioned for males, such as the precocious age of the rabbits that supplied the pituitary or the incompatibility of the rabbit's gonadotrophins administered to fish.

The BCPE proved to be efficient in more than 70% of the induced females. The high percentage attests its potentiality when compared to the action of other hormones, as described in Romagosa *et al.* (1990), who induced *P. mesopotamicus* females using hCG and salmon pituitary extract, obtaining 57.9 and 84% of efficacy, respectively. Amaral Jr. *et al.* (1996) obtained 90%, employing carp extract on *Cyprinus carpio,* Silva *et al.* (1997) obtained 100% of response working with BCPE in curimbatás. The high variability of numbers is due to differences among species and to the material used for induction.

Amaral Jr. (1995) stated that avian pituitary hormones are chemically similar to teleosts hormones. In addition, the aminoacids sequences of the hypothalamic hormones are very similar among fish and birds. According to Donaldson (1996), there are two or three distinct forms of GnRH in various fish species. Yu *et al.* (1995) tested pituitary

extract of broiler chicken, duck, turkey, ostrich, and goose on the spawning of *Paramisgurnus dabryanus*, *Misgurnus anguillicaudutus,* and *Hypophthalmichthys molitrix*. They reported good results from the first two inducers, in which laboratorial tests indicated the FSH presence, which certainly acts as GTH (gonadotrophin hormone) on the stimulation of estradiol-17 (in vitro) production. As Barroso (1999) stated, the *P. mesopotamicus* had a positive reaction to BCPE. This study showed good results for BCPE as gonadal inducer, promoting spawn to satisfactory results and showing promising perspectives for BCPE.

No difference (P>0.05) concerning the variable ATU was verified between CPE and BCPE treatments (Table 3), although a tendency for higher average in animals induced with BCPE was noticed. In great part of the experiment, it was observed that animals induced by BCPE took longer to spawn, reaching 291 ATU, whereas the animals treated with CPE did not exceed 263 ATU. This ATU difference between CPE and BCPE treatments, in water at the same temperature, allows the supposition that BCPE gonadotropins take longer to be absorbed, enter the bloodstream and act in the gonads of female *P. mesopotamicus*. Godinho and Godinho (1986) induced the same species to spawning by administering CPE and observed an ATU of 325 and 289 after the second injection at a temperature that ranged from 22 to 24.5°C. The ATU results of the CPE experiment were reasonably lower, due to the average temperature of 25.5°C. Among other factors, the ATU value depends mainly on the water temperature in the tank. This statement is reinforced by Lam (1983), who said that temperature was the most important factor at spawning control.

The ATU varies according to the species and the inducers used, as in Lee *et al.* (1988) experiments, where the authors reported 328 of ATU in the mullet (*Mugil cephalus*), using CPE and 464.4 ATU, with LhRH-a (analog Luteinizing Released Hormones). Kucharczyk *et al.* (1998) reported ATU of 230 to 348, from the application of the second dose until the spawning, when studying the use of a mixture of FSH+LH associated with pimozide or methoclopramide (Sigma Chemical Co., St. Louis, MO, USA) for spawning and sperm production of perch (*Perca fluviatilis*). During this study, it was observed that it took longer for the animals induced with BCPE to spawn, reaching up to 291 ATU; whereas this value did not go over 263 ATU for the ones treated with CPE.

The average number of oocytes per gram of ova did not differ (P>0.05) from the animals treated with CPE and with BCPE (Table 3). Ceccarelli *et al.* (2000) stated that the expected number of oocytes per gram of ova for *P. mesopotamicus* is 1200. In this experiment, the number was, in average, 20% higher. This number varies according to the species, as verified by Sato *et al.* (1996) who treated white piau (*Schizodon knerii*) with pituitary extracts and obtained 930 oocytes per gram of ova. This variable is directly linked to the animal size and its species (Ceccarelli *et al.*, 2000). However, Lovell (1989), who studied canal catfish females (*Ictalurus punctatus*), stated that reproductive characteristics such as: number of eggs, egg size, spawning rate, and larvae quality are directly related to nutritional condition. Such information may lead to the belief that pituitary extract does not affect the number oocytes/gram of spawning, since in this study the values were identical in all treatments, although there were no non-induced animals to compare with.

Fertilization and hatching rate from CPE and BCPE treatments proved to be similar (P>0.05) (Table 3). Fertilization rate and hatching rates might be directly linked to feeding. Watanabe (1985) affirmed that an adequate diet for breeders might improve spawning quality as well as the produced eggs, resulting in healthy, good-quality fingerlings. Brzuska and Grzywaczewski (1999) induced carps (*Cyprinus carpio*) from two different lineages, using CPE and pelletized GnRH, obtaining a fertilization index of 93%. Bernardino *et al.* (1993) induced the matrinchã (*Brycon cephalus*) using CPE and observed an average of 85% in fertilization. Sato *et al.* (1996) also employed CPE on white piau (*Scizodon knerii*) to induce spawning obtaining 69% of fertilization. Rezende *et al.* (1996) induced the *Leporinus elongatus* resulting in a 73% fertilization rate. This study showed low fertilization rates (57% and 49.6%) and hatching rates (57% and 46.9%) – for animals induced by CPE and BCPE, respectively. These figures may suggest that the origin of the pituitary extract was a determining effect on these rates. On the other hand, factors enumerated by Bromage (1995) such as the quality of breeders feeding, environmental conditions, stress at capture, manipulation in the induction tank, genetics, incubation conditions and maturity, among others, may affect the rates.

Conclusion

The results of this study show that broiler chicken pituitary extract, when compared to carp pituitary extract, proved to be effective in inducing spawning and sperm production. On the other hand, rabbit pituitary extract did not respond positively as a good gonodal inducer on males and females *P. mesopotamicus*. Semen analysis from animals treated with broiler chicken pituitary extract and carp pituitary extract showed similar quality. According to these results, new studies are recommended, especially the ones related to the age of rabbit pituitary extract.

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