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EFFECT OF DIFFERENT FEEDING REGIMES ON REPRODUCTIVE PERFORMANCE AND LARVAE PRODUCTION OF BLUE TILAPIA (*Oreochromis aureus*) BREEDERS.

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ABSTRACT

Effect of different feeding regimes, depending on fed fat levels and/or protein energy ratio (P:E), during rest and ovulation phases, was investigated, in the present study, on reproductive performance and larvae production of blue Tilapia (*Oreochromis aureus*) breeders. Two diets were formulated and supplemented to experimental fish groups; the first fish group was supplemented feed (I), low fat diet (4.56%), (protein: energy ratio: 67 mg/kcal), during ovulation phase, then feed (II), rich fat diet (9.13%), (P:E ratio: 58.4 mg/kcal) was supplemented to the same fish group in resting phase as long as 7 days. The second fish group was supplemented with feed (II) only allover the experimental period extended for 112 day.

The results revealed that there is a considerable difference (P?0.05) in food and nutrients requirements before and after fish broodstock ovulation. The best egg and larvae production (12184 egg/kg female) was obtained by using the first feeding regime; feed (I), after the beginning of ovulation phase, and then feed (II) supplementation in the rest phase. In the second experimental fish group, females were fed allover the experimental period, fat rich diet (9.13%) only (second feeding regime). Egg production in that group was observed to be less (8953 eggs/kg female) than the first group. This result may be attributed to fat accumulation on the internal organs, especially ovaries that hinder normal process of reproduction.

It could be concluded that not only feeding of spawners plays a considerable role in reproductive performance, but also stage of spawning feeding requirements is important in operating succeeding spawning.

Key words: blue tilapia, feeding regime, reproductive performance, larvae production.

INTRODUCTION

Fish is expected to be considering the main food for the world population in the next decade; especially Tilapias, the major protein source in many developing countries. In Egypt, GAFRD (2002) reported that the most common species of fish produced are tilapias (44.58% of total fish farming production). The traditional Egyptian fish farms depend mostly, in stocking Tilapia fry, on hatcheries and wild fry in the northern lakes and River Nile. The numbers and species of harvested fry vary, quantitatively and qualitatively, from season to another and the fry marketing retail prices are relatively fluctuated, thereby, the need of developing and controlling Tilapia broodstock reproductive performance becomes increasingly urgent and important.

The propagation of fishes has been practiced from time immemorial in different parts of the world. Techniques for production of adequate quantities of high quality fish seed from captive broodstock are necessary for large-scale expansion of aquaculture. Feeding broodstock plays an important role in applying these techniques for marine or freshwater fish hatcheries. In the case of *Oreochromis aureus*, the female does not eat during the incubation period because it belongs to breeder groups (Macintosh and Little 1995); broodstock dietary requirements ensuring and becomes a must.

In raising brood fish, care has to be taken not only to select the best strains but also adequate food, quantitatively and qualitatively, must be given to ensure proper development of the gonads. De Silva and Anderson (1995) reported that nutrition is known to affect reproductive success in fishes. During gametogenesis, female fish require a food richer than usual in proteins and lipids to produce the vitellogenin, which is progressively stored as yolk in the oocytes. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a successful reproduction. Nickolsky (1962) stated that physiological condition of fish depends mainly upon three factors, genetically, environmental and nutritional factors. Therefore, unfavorable broodstock's nutritional conditions may cause poor fecundity and fertility, deformed embryos, week larvae, where females produce imperfect eggs and the males may produce low-grade quality of milt (Watanabe *et al.*, 1984; Kanazawa, 1985; Omar, 1986; Rose, 1990; El-Ebiary, 1994 and Mabrouk, 2003).

Because of being cold-blooded animal, fish do not have to expend energy in order to maintain body temperature; therefore, more energy is available to the fish for growth, activity and reproduction (NRC, 1977).

Fat sterols are polycyclic compounds, long-chain alcohols and function as components of several hormone systems, especially in sexual maturation and sex-related physiological functions (FAO, 1980). Dietary lipids have two main functions: 1) as a source of energy, and 2) as a source of fatty acids. Lipids are also important factors in the palatability of feeds (Michael, 1987). Lipids are the best source of energy for fish, followed by proteins and the carbohydrates, respectively and it exert a greater protein sparing effect for serving as alternative energy source, thereby reducing the proportion of dietary protein that must be catabolized in order to meet energy demand (Simon Wilkinson, 2003). Excess or insufficient dietary energy levels result in reduced fish growth rates and/or reproductive performance. Excess energy content will inhibit food intake and also reduce the protein available for growth and insufficient dietary energy lead to protein breakdown. Energy needs for maintenance and movement will be fulfilled before energy is used for growth and/or reproduction, thus, if the protein/ energy ratio is too low, protein will be used to satisfy energy requirements first; what is left will be available for reproduction and growth (NRC, 1983, Michael, 1987 and Wendy *et al.*, 1998).

Therefore, the aim of the present study was to evaluate the effects of different feeding regimes, depending on fat level, and/ or P:E ratio on reproductive performance and larvae production of blue Tilapia (*Oreochromis aureus*) broodtocks.

MATERIALS AND METHODS

The present work was undertaken at El-Magharaby Tilapia Production Private Hatchery, Kafr El-Shiekh Governorate, Egypt.

The present experiments were carried out with blue Tilapia (*Oreochromis aureus*) females of 6-8 months old of the same spawn, which achieved sexual maturity under pond farm management conditions. Blue Tilapia females were transported and stocked from fishponds into concrete basins (2.5 m^3 water volume) in April 20, 2002 to spawn under natural photoperiod. The water flow of 1-2 lit. /min. was secured and provided by a sprinkler hanging above the basin. Average water temperature in the spawning basins ranged from 23 to 28°C during the experimental period.

Oreochromis aureus breeders were divided into two groups (Two replicates spawning basins per group). Fish in each group were fed with floating pellets of different composition, according to investigation requirements, at a rate of 1.5% of the total fish body weight per day, as follows:

First group:

Consisted of two concrete basins, eight females were stocked in each basin with four males [2:1 sex ratio, female to male, Hughes and Behrends (1983)]. This group of broodstock fish received two types of diet in April 22, 2002, the first diet was spawning diet (Feed I), received during spawning period, low fat content diet (4.56%). The second diet was resting diet (Feed II), received after each spawning batch for 7 days, rich in fat (9.13%). From the eighth day to the time of next spawning batch, the fish fed feed (I) again and so on for 112 days (16 week).

Second group:

Consisted of two concrete basins, eight females were stocked in each basin with four males (sex ratio 2:1, female to male). The fish of this group were supplemented one type of feed only (Feed II), rich in fat (9.13%), for 112 days (16 week).

Composition and chemical analysis of experimental diets (Feed I and II) are shown in Table (1).

Females (average body weight of 80.0 g) and males (105.0 g) of comparable weight, were stocked together in each basin to minimize injures caused by the aggressive behavior of the males. The spawning was checked daily and the eggs were removed from female mouth brooders one day post spawning. The eggs were counted, sample of 100 eggs were measured and then incubated in funnel type to be hatched. Incubators made from 1.5 liter clear plastic bottle. The number of eggs per incubator was 400-500 (Essa, 1995). Only slow water flow was provided in the incubators (0.3 lit. /min.), at hatching stage, the water flow was gradually increased (about 1.0 lit. /min.) until the end of hatching.

During eggs incubation period, the following parameters were calculated:

1) Fecundity: was defined as the number of eggs produced/ kg body weight of each female according to Smitherman *et al.* (1988).
2) Time required for embryonic development in hours.
3) Hatching rate=the number of live larvae/total number of eggs sample x 100
4) Dead embryos and larvae were counted and removed daily

Hatching larvae were allowed to remain in the incubators until yolk sac absorption was completed at six to seven days after hatching.

Chemical analysis of diets and fish were analyzed according to procedures of AOAC (1985). Digestible Energy (DE) was calculated according to Aquaculture Development and Coordination Programme (ADCP, 1983) on dry matter basis.

One-way analysis of variance (ANOVA) was used to obtain feeding regime and experimental error effects according to Snedecor and Cochran (1967).

Table (1): Composition and proximate analysis of the experimental diets (% wet DM)

INGREDIENT	FEED I	FEED II
Fish meal	17.5	18.0
Blood meal	5.0	5.0
Soybean meal	20.0	20.0
Wheat bran	2.5	2.3
Rice bran	23.0	20.0
Wheat milling by-product	10.5	10.2
Cotton seed cake	15.0	15.0
Bone meal	1.2	1.2
Yeast	2.0	2.0
Oil	2.0	5.0
Vit. And mineral mix.	1.3	1.3
Total	100.0	100.0
Proximate analysis (%Wet weight)		
Moisture	13.17	11.05
Crude protein	25.43	25.20
Lipid	4.56	9.13
Carbohydrates	46.93	49.52
Ash	9.91	5.10
Total	100.0	100.0
GE*	379.3	431.8
P:E (mg/kcal)	67.0	58.4
DE (kcal/kg)**	2739.0	3173.6

***Gross energy (kcal/100 g feed)** ****According to ADCP (1983).**

RESULTS AND DISCUSSION

Within the water temperature range of 20-28°C, blue Tilapia (*Oreochromis aureus*) females of the first group spawned 8 times in intervals as short as two weeks within 112 days. They fed with feed I (low in fat content, 4.56%) before and during spawning activity, then fed diet II (rich in fat content, 9.13%) directly after spawning for 7 days. Breeders of the second group (fed diet II all the time) were spawned 6 times within 112 days in intervals approximately 18 days. Aureli (1988); Essa and Salama (1994) reported that female Tilapia can spawn in intervals as short as two weeks if they are not allowed to incubate the eggs.

Hatching rate

Fish of the first group produced 26.5% more eggs/female than those of the second group. Therefore, the mean production of eggs/kg of female body weight of first group (12184 eggs) was significantly (P?0.05) higher than those of the second group (8953 eggs) (Table 2). Thus the increased spawning frequency of the first group females would enable more efficient production of greater number of eggs. Santiago *et al.* (1983) reported that *O. niloticus* females fed varying protein diets of 20 to 50% did not show significant differences in the mean number of eggs per one spawn. This results are in agreement with the present study, where protein content in both experimental feeds were 25.43% and 25.20% for feed (I) and feed (II), respectively. Therefore, it could be concluded that dietary fat requirement of *Oreochromis aureus* is correlated with spawning phase, where it decreased during ovulation phase to 4.56% and increased up to 9.13% during the rest phase.

Table (2): Means of reproductive traits of blue Tilapia (*Oreochromis aureus*) spawned under different feeding regimes

Item	First feeding regime	Second feeding regime
Female weight	80.00	80.00
No. of eggs/kg female*	12184[a]	8953[b]
No. of spawns measured	8	6
Egg length (mm)	2.00	1.90
Egg width (mm)	1.43	1.42
Time required for developing eggs (hour)	70-90	70-90
Hatch (%)	85.00	83.90
Newly hatched larvae length (mm)	5.20	5.10

***Means with different superscripts differ significantly (P?0.05)**

Table (3): Proximate composition of fish breeders over the experimental period (112 days)

Item	Initial	After 112 days (% wet weight)	
		First group	Second group
Moisture (%)	78.37	78.82	77.40
Crude protein (%)	12.91	14.07	14.12
Crude fat (%)	4.16	4.11	8.32
Ash (%)	4.56	3.00	2.16

Fry production

Results of the young fry production trials under two feeding regimes are shown in Table (4). The highest total larvae production (P?0.05) expressed in terms of larvae/group and larvae/g female were found in the first group females that spawned under the first feeding regime. Total larvae production per gram female for the first group was 12.19 larvae, while that of the second group was 8.95 larvae. Total larvae production per group (8 females) during 112-day study (16 week) ranged from 5730 larvae for the second group to 7798 larvae for the first group. Larvae production gradually increased through the sixth and seventh clutch, when all treatments reached maximum larvae production (Table 4).

The larger variation in larvae from individual group at each clutch may be resulted from a combination of factors: variability in fecundity of individual females (Mires, 1982), differences in spawning time (Essa, 1995), and the relative asynchrony of spawning cycles between individual females (Jalabert and Zohar, 1982). Variation between the experimental feeding groups may be attributed also to difference between fish metabolic rate during ovulation and resting phases.

Table (4): Effect of feeding regime on production of newly hatched fry (*Oreochromis aureus*) hatched during 6-8 clutches for 112 days.

No. of clutches	Treatments			
	First feeding regime		Second feeding regime	
	Total larvae/group*	No. of larvae/g female	Total larvae/group*	No. of larvae/g female
1	127 ± 54	0.20	107 ± 23	0.17
2	849 ± 195	1.33	431 ± 111	0.67
3	1062 ± 217	1.66	569 ± 239	0.89
4	1225 ± 413	1.91	1049 ± 429	1.64
5	1294 ± 419	2.02	------	------
6	1354 ± 501	2.12	1698 ± 595	2.65
7	1491 ± 570	2.33	1876 ±563	2.93
8	396 ± 129	0.62	------	------
Total larvae**	7798^a	12.19	5730^b	8.95

* One group = 8 females + 4 males

** Means not followed by the same letter are different (P?0.05).

Growth rate

Winfree (1979) reported that dietary protein and energy levels resulting in highest growth rates in *Oreochromis aureus* were 34-56% (Crude protein), 3200-4600 kcal/kg (gross dietary energy) and 115 mg/kcal (protein energy ratio). Comparing with Winfree (1979) results, fish in the present study requires sufficient energy for adequate building up gametes not for growth and fattening, so fish broodstock has exposed to different levels of fats and lower protein energy ratio (67 and 58.4 mg/kcal for feed I and II, respectively) owing to activity of spawning stage. These lowered protein energy ratios, in the present study's diets, could be attributed to either low protein content (25.43% and 25.2%) or high gross energy content (379.3 and 431.8 kcal/100 g). Thereafter, feeding broodstock needs more research plans to answer many questions about the optimal protein energy ratio for broodstock fish species.

One major explanation for the high protein requirements in fish is that they utilize a significant portion of the dietary protein to meet their energy needs (Cowey and Luquet, 1983). There is increasing evidence that the dietary protein supply can be considerable reduced in the diets of teleosts based on DP/DE ratios and high-energy diets with low DP/DE ratios proven to be beneficial in terms of feed efficiency, protein sparing and for effectively reducing organic matter and nitrogen loss (Cho and Kaushik, 1990; Kaushik and Médale, 1994).

Chemical composition

Results of the chemical composition of fish breeders (Table 3) indicated that the least deposition of fat was recorded in the fish fed the first feeding regime, which had the lower fat content (4.11%), while it was 8.32% in the fish fed the second feeding regime. This could be correlated with the high concentration of fat content in feed II (Table 1). In agreement with the present results, Horvath (1981); Pathmasothy (1985) and Springate *et al.*, (1985) reported that high fat diet fed to females ready for ovulation will cause fatness which would relatively reduce normal process of reproduction. According to many nutrition workers, higher nutritive value diets tended to give larger ovaries and fecundity for Tilapias (Santiago *et al.*, 1981 and Essa, 1993), rainbow trout, *Salmo gairderi* (Springate *et al.*, 1985) and *Leptobarbus hoevenii* (Pathmasothy, 1985). On the other hand, no differences were found in egg length, egg width, percentage hatch or newly hatched larvae length between the two feeding regimes (Table 2). Pathmasothy (1985) found the same results for *Leptobarbus hoevenii.*

In the first feeding regime, it apparent that feed (I), less in fat (4.56%) (P:E ratio 67 mg/kcal), given to females ready for ovulation prevents unnatural fatness of ovaries, which means that a part of dietary protein will be degraded to supply energy requirement under low fat condition. Feed (II), rich in fat (9.13%) (P:E ratio 58.4 mg/kcal), provide more efficient source of energy which would highly promote the vitellogensis with ovulated ovaries and also sparing protein for building up sexual products (eggs or milt) in the gonads which start soon after the previous spawning. In agreement with the present study, Bruce and Elizabeth (1999) stated that fish broodstock consumed more protein than lipid for nutritional requirements in the ovulation phase. Hajra *et al.* (1988) reported that variation away from the optimum ratio between protein/ energy will result in either the catabolism of protein for energy, or the production of fatty animals and both scenarios result in sub optimal feed efficiency. Therefore, energy: protein ratio of the broodstock diets could be a major determinant of spawning success and larval quality.

In summary, not only feeding of spawners plays a considerable role in reproductive performance, but also stage of spawning feeding requirements is important in operating succeeding spawning. Excellent egg and larvae production can be occurred using the following feeding regimes: shortly after the spawning when the ovary of *Oreochromis aureus* females is empty, a fat and carbohydrates rich diet should be used to accelerate the egg development. In that time the energy accumulates in the ovary. After the completed vitellogensis, females need more protein rich food and moderate dietary fat content to prevent the fattening of the organs, especially ovaries, which would relatively reduce normal process of reproduction.

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تأثير نظم التغذية المختلفة على بعض معايير التناسل وأنتاج اليرقات لأمهات أسماك البلطى الحسانى (الأزرق)

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أجرى هذا البحث من أجل دراسة تأثير نظم التغذية المختلفة على الكفاءة التناسلية وإنتاج اليرقات لأسماك البلطى الحسانى (البلطى الأزرق). وقد قسمت الأسماك إلى مجموعتين تجريبيتين الأولى قدم لها نوعين من الأعلاف هما العلف رقم (١) طوال فترة التبويض وهو علف منخفض فى قيمة الدهون (٤,٥٦%) وكانت نسبة البروتين الى الطاقة فيه ٦٧ مجم بروتين / كيلو كالورى، بينما قدم إلى نفس المجموعة من الأسماك بعد انتهاء التفريخ وأثناء فترة الراحة ولمدة ٧ أيام علف رقم (٢) الغنى فى قيمة الدهون (٩,١٣%) وكانت نسبة البروتين الى الطاقة فيه ٥٨,٤ مجم بروتين / كيلو كالورى ، أما المجموعة الثانية من الأسماك فقد تناولت العلف رقم (٢) فقط طوال فترة التجربة.

أثبتت النتائج أن هناك اختلافات كبيرة فى الاحتياجات الغذائية لأمهات أسماك البلطى الحسانى قبل وبعد التبويض، وأفضل النتائج المتحصل عليها فى إنتاج البويضات واليرقات من أمهات أسماك البلطى الحسانى تم الحصول عليها عند استخدام النظام الغذائى الأول (١٢١٨٤ بويضة / كجم أم) حيث تناولت الأسماك فى مرحلة التبويض العلف رقم (١) المنخفض فى محتواه من الدهون (٤,٥٦%) وذلك لمنع تراكم الدهون على المناسل فى هذه المرحلة حتى لا تعوق عملية التبويض، بينما غذيت الأسماك فى مرحلة الراحة والأعداد لمرحلة التبويض التالية على العلف رقم (٢) الغنى فى محتواه من الدهون (٩,١٣%) من أجل بناء الخلايا التناسلية وتكوين المح، وعندما غذيت الأمهات على عليقة واحدة (النظام الغذائى الثانى) الغنية بالدهون (٩,١٣%) طول الوقت أدى هذا النظام الغذائى إلى انخفاض عدد البويضات إلى ٨٩٥٣ بويضة / كجم أم وقد تعزى هذه النتيجة إلى أنخفاض نسبة البروتين الى الطاقة (٥٨,٤ مجم بروتين/ كيلو كالورى) مما يؤدى الى تراكم الدهون على الأعضاء الداخلية، خاصة المناسل وبالتالى انخفاض كفاءة التفريخ لدى الأمهات.

لذلك ينصح باستخدام نسبة دهون منخفضة (٤,٥٦%) فى تغذية أمهات البلطى الحسانى أثناء عملية التبويض وزيادة هذه النسبة إلى (٩,١٣%) أثناء فترة الراحة والأعداد لمرحلة جديدة، وهذا يعتبر مؤشر الى أن أهمية تغذية الأمهات لا تنحصر فقط فى جودة مواصفات الأعلاف (كما ونوعا) المقدمة اليها بل أيضا فى الأحتياجات الغذائية للأمهات تبعا للمرحلة الفسيولوجية التى تمر بها أثناء دورة التفريخ. ونظرا لأن نسبة البروتين الى الطاقة قد يكون لها دور مؤثر فى نجاح عملية التفريخ وجودة اليرقات الناتجة، فأن هذا الأتزان الغذائى يحتاج الى مزيد من البحث.