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Growth Performance, Sex Hormone Levels and Maturation Ability of Pla Pho (*Pangasius bocourti*) Fed with *Spirulina* Supplementary Pellet and Hormone Application

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ABSTRACT

The purpose of this study was to evaluate the effects of *Spirulina platensis* (SP) supplementary pellet feed and hormone application to Pla Pho (*Pangasius bocourti*) brood stock on growth sex hormone level and maturation abilities. The study was divided into three experiments. In experiment-1, the treatment 2 (3% of SP) of the Northeast population had a difference compared with the control. The average sex hormone (estradiol) levels from two collections in North and Northeast catfish fed with 3% SP were higher than in other treatments, while the average estradiol levels in March were higher than in January. Moreover, in June and July, 3 males and 2 females brood stock from 3% of SP feeding gave sperms and eggs, while only sperm were obtained from other treatments. In experiment-2, in Northeast population, estradiol was highest from catfish fed with 3% SP in April and gave more mature fish than control treatment in June and August. In experimental 3, the North population fish, four months later (in July), hormone was injected and implanted highest testosterone level and number of mature male and female fish were obtained in July and August. In conclusion, 3% *Spirulina* supplemented in pellet feed and hormone application improved growth and maturation performance of brood stock *Pangasius* catfish. Therefore, *Spirulina* supplemented feed and hormone application is recommended for *P. bocourti* farmers.

Key Words: *Pangasius bocourti;* Brood stock; *Spirulina platensis* (SP); Hormone treatment

INTRODUCTION

At present, the aquaculture industry has become more important in the Asian Region, including the Mekong River. Catfish of the family Pangasiidae are commercially produced throughout the region. Indigenous species including the Mekong giant catfish (*Pangasianodon gigas*) Sutchi catfish (*Pangasianodon hypophthalmus*) and Pla pho (*Pangasius bocourti*) are economical freshwater fish species. Pla Pho is also known as "basa fish", a type of catfish native to the Mekong river delta in Vietnam and the Chao Phraya basin in Thailand. It is recognized as one of the greatest food fishes in the international market because of its white fillet and high nutritional values. This catfish is widely cultured in floating cages in the Mekong delta. Breeding in captivity was first achieved in 1995 and artificial propagation was later obtained with broods reared both in ponds and in floating cages (Cacot, 1999). A problem with breeding in captivity has been the late sexual maturity and stunted mass production. The brood stock needs to be improved in terms of growth, maturation, fecundity of eggs, sperm and fingerling mass production. Such improvements can be induced by environmental manipulation such as fish feed and or exogenous hormone administration. Hormonal induction of spawning is necessary for species that do not spawn spontaneously in captivity.

Among the microalgae used as foodstuffs, food supplements and animal feed in many parts of the world, *Spirulina* spp. is the most popular due to high nutrient values and cost effectiveness at the farm scale (Anupama, 2000). SP is an excellent source of gamma linolenic acid (GLA, ~1%), an essential polyunsaturated fatty acid. This essential fatty acid is a precursor for the body's prostaglandins (PGE1), the master hormones that control many body functions. PGE1 is involved in many tasks including the regulation of blood pressure, cholesterol synthesis, inflammation and cell proliferation. PGE1 is usually formed dietary linolenic acid and the GLA is transformed to PGE1.

Maturation is under the hypothalamus gonadal axis control. Gonadotropin releasing hormone (GnRH) stimulates luteinizing hormone (LH or GTH II) release by the hypophysis (Peter & Yu, 1997), while it stimulates the maturation competence of gametogenesis and releases of the maturation inducing steroid (MIS) from the gonad, inducing final maturation (Nagayama, 1994). Therefore, the application of luteinizing hormone-releasing hormone (LHRHa) has been used to induce maturation and spawning in many fish species (Zohar, 1988).

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This study was aimed at evaluating *Spirulina* supplements and hormone administration by measuring the weight gain, sex hormone production and the number of ready-to-breed brood stocks of *P. bocourti*.

MATERIALS AND METHODS

Brood Stock, Treatment and Feed Preparation

Experiment 1. A total of 60 *P. bocourti* (2 & 3 years old of the northeast population from Nakornpanum Fishery Center and the north population from Chiang-Rai Fishery Center; Fig. 1) with an average weight of 1.2 kg were randomly treated by one of four treatments for 11 months (September 2006-July 2007). Three and two replicates of the North and Northeast population with three fish per replicate were performed. All pellet feed formulations comprised approximately 30% crude protein level prior to incorporation of fresh SP. Fish were fed daily with pellets at equal to 2% of the body weight. The stocking rate was 1 fish/3 m^2. The different treatments were performed in one earthen pond with each treatment separated by nylon net. Treatment 1 was pellet without SP (control), while treatments 2, 3 and 4 were pellets added with fresh SP at the rate of 3, 6 and 9% of wet weight, respectively. The compositions of each pellets (Table I) was determined using approximate analysis (AOAC, 1990).

Experiment 2. Treatment 1 (T1) was formulated pellet without SP (control), while treatments 2 and 3 were added with fresh SP at an amount of 3 and 6% (wet weight), respectively. For this treatment we used the northeast population of fish (1.6 kg) and each treatment contained 3 females and 3 males as replication and catfish were cultured for 14 months (September 2006-November 2007).

Experiment 3. A total of 18 *P. bocourti* from the northern population, with an average weight of 1.3 kg were randomly treated by one of three treatments for four months (April–July, 2008). Treatment 1, injected 0.9% NaCl solution as a control; Treatment 2, injected hormone (LHRHa) 50 ug kg^{-1} body weight (BW) three intramuscular injections once per month for 3 months and treatment 3 implanted hormone (LHRHa)150 ug/kg BW in silastic tube and covered by elastomer for sustained releases for 3 months (Lee *et al.*, 1986). Each treatment contained 6 fish kept in one earthen pond.

Data sampling. Gain in weight (GW), feed conversion rate (FCR) and biomass were examined on monthly basis. One mL of blood sample was taken twice from caudal vasculature of each fish (Fig. 4). Serum samples were separated by centrifugation at 10,000 rpm for 5 min and stored at -20°C until analysis. A 50 µL of serum was sent to the Faculty of Medicine, Chiang Mai University for estradiol and testosterone quantification by Electro-chemiluminescent assay (Elecsys1010, Roche, Germany). In June, July and August, the fish brood stocks were checked by sampling sperms and eggs (Fig. 5).

Statistical analysis. The variation of treatment was analyzed by analysis of variance (ANOVA, $P<0.05$). Significant differences between treatments were analyzed using Duncans multiple range (DMR) test by SPSS version 15.

Fig. 1. Gained in weight of *P. bocourti* populations from each treatment during September 2006–March 2007

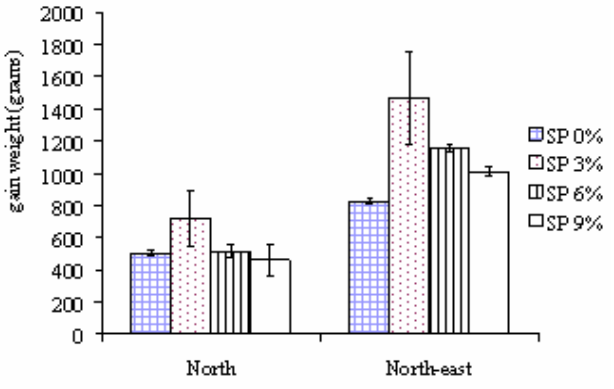


Fig. 2. Sex hormone (estradiol) levels of *P. bocourti* from each treatment in September 2006–March 2007

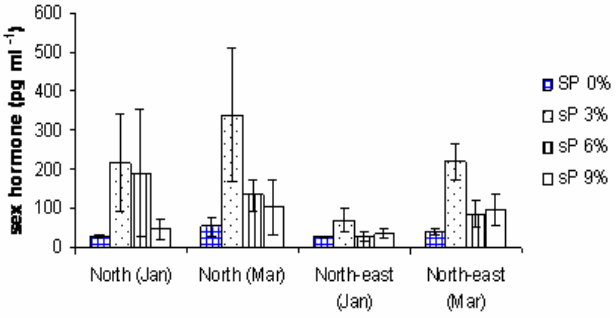
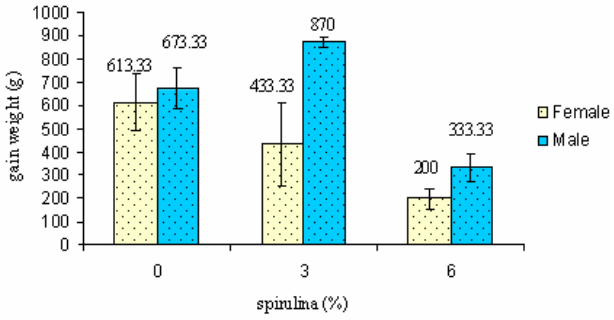


Fig. 3. Gain weight (g) of female and male *P. bocourti* northeast population during September 2007–May 2008



RESULTS

Experiment 1. A nutrient composition from each treatment in term of total crude protein were not significantly different ($P>0.05$). However, there were significant differences in fat, nitrogen and energy contents when SP and control treatments were compared (Table I). The gain in weight of

Table I. Nutrient content of the treatment feeds

Treatment	Dry Matter						
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Nitrogen free extract (%)	Energy (kJ kg^{-1})
Control	0.06±0.05^{a}	10.19±0.02^{a}	27.59±1.32^{a}	2.69±0.01^{a}	12.94±0.07^{b}	46.55±1.21^{b}	1.36±0.02^{a}
3% SP	0.26±0.01^{c}	10.75±0.14^{b}	29.22±0.43^{a}	6.42±0.32^{b}	10.05±0.25^{a}	43.43±0.98^{a}	1.45±0.01^{b}
6% SP	0.25±0.01^{c}	10.09±0.20^{a}	27.61±1.26^{a}	8.22±0.09^{c}	15.04±0.18^{c}	38.92±1.41^{a}	1.42±0.00^{b}
9% SP	0.20±0.01^{b}	10.05±0.05^{a}	28.73±0.61^{a}	7.13±0.03^{b}	13.72±0.89^{b}	40.27±0.35^{a}	1.42±0.00^{b}

*Means that do not share the same letter superscripts in the same column are statistically significant (P<0.05.); a, b and c significance different among treatments mean; *Spirulina platensis* (SP)

fish from treatment with SP was higher than treatment control in northeast population group (Fig. 1). However, there was no significant difference in the estradiol level from both population, which had a trend to be higher than 3% SP in March and January (Fig. 2). Moreover, a number of mature fishes in June and July 2007, was also higher with 3% SP compared to other treatments (Table II).

Experiment 2. The gain in weight of fish from all treatments of male group was higher than female group and treatment with 3% SP. However, it was highest in treatment control in female group but non-significantly different (Fig. 3). The testosterone and estradiol level from male and female were higher in April than in November from all treatments. Moreover, estradiol level from treatment with SP was higher than control in April (Fig. 4 & 5). In addition, a number of mature fish from June to August 2008 was also highest in treatment with 3 and 6% SP, respectively (Table III).

Experiment 3. Fish with hormone injection had the highest weight gain, although not significant (Fig. 6). The testosterone level in July after hormone application was highest in treatment with hormone implant (Fig. 7). Moreover, the number of mature fish in August 2008 was also higher. The treatment with hormone injection had 60% fertilization rate (Table IV).

DISCUSSION

In general, fish feed incorporated with SP resulted in higher weight, sex hormone levels and number of mature fish than fish feed without SP. Because of nutrient composition from treatment with SP in term of total crude protein was highest (29.22%) of protein content in pellet feed was from treatment with 3% SP. Moreover, there was significant difference between treatment group with SP and without SP (control) in term of fat, nitrogen and energy contents. In addition, SP is a high quality food with high levels of protein (55-70% on dry weight basis), vitamins such as B, C and E, minerals, polyunsaturated fatty acids, zeaxanthin and myxoxanthophyll. Since estradiol controls female reproductive function in all classes of vertebrates and is responsible for vitellogenesis in oviparous species, it is not surprising that together with increased plasma estradiol, enhanced ovarian growth was noted in catfish fed with SP supplementary diets. As estradiol increases plasma ovarian steroid level, the observed general increase in more mature fish (Nagayama, 1994). Other studies suggested that

Table II. Number of brood stock from northeast and north population with sperm and egg collection in June and July 2007

Treatment	Amount	Male	Female	Sperm	Egg
No SP	6	3	3	3	-
SP 3%	6	3	3	3	2
SP 6%	6	3	3	3	-
SP 9%	6	3	3	3	-

Fig. 4. Testosterone (ng/mL) of male *P. bocourti* northeast population in November 2007 and April 2008

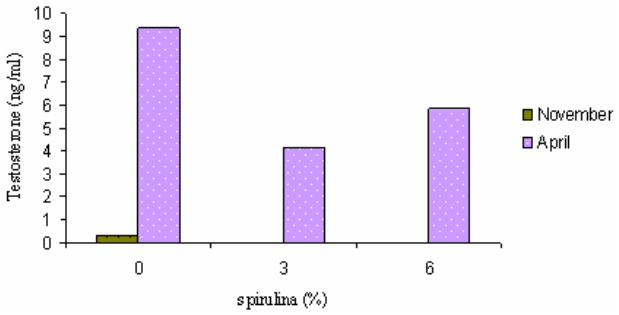
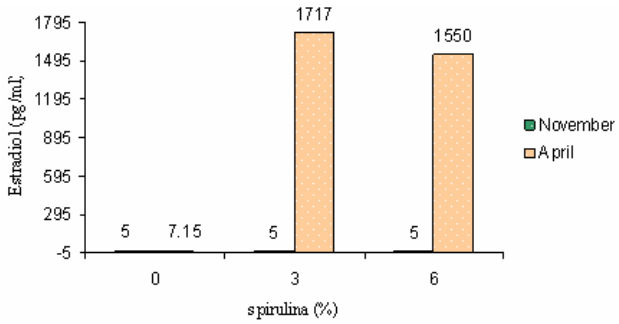


Fig. 5. Estradiol (pg/ml) of female *P. bocourti* northeast population in November 2007 and April 2008



Spirulina supplement in the feed of freshwater fish has an effect of improving growth and promoting gonad development and maturation (Yamaguchi, 1980).

In Experiment 1 sperm production seemed to be un-affected by SP. This may be since GLA is not linked to this synthesis. Moreover, similar results were also observed among the successive progenies (F_1 & F_2). Significant differences in the fatty acid profile of eggs were observed between the raw *Spirulina* and the commercial diet groups, with raw *Spirulina* group containing more linoleic acid, γ-linolenic acid and Σn-6 highly un-saturated fatty acids

Table III. Number of brood stock from northeast strain with sperm and egg collection

Treatment	Amount	Average weight (kg)	Egg (g kg^{-1})	No. of eggs g^{-1}	Sperm (mL kg^{-1})	Number of sperms mL^{-1}	Sampling date
T1(no SP)	2	1.4	-	-	5.74	-	July 08
T2 (SP 3%)	1	1.7	29.41	304	-	-	June 08
	2	1.6	-	-	3.75	297.06 $x10^6$	June,Aug 08
T3 (SP 6%)	5	1.4	-	-	1.6	-	June,Aug 08

Table IV. Effects of LHRHa hormone treatment on egg and sperm production

Treatment	Number	Average weight (kg)	Sperms collected (mL kg^{-1})	Amount (sperm mL^{-1})	No. of egg g^{-1}	Eggs (g kg^{-1})	Fertilization rate (%)	Sampling date
T1 (NaCl)	0	0	0	0	0	0	0	0
T2 (Inject	1	1.6	0	0	310	25	60	14Aug 08
hormone)	1	2.3	0	0	302	17.39	60	
	1	1.5	0.53	305.16 x 10^6	0	0	0	14Aug 08
T3 (Implant	1	1.5	3.00	331.32 x 10^6	0	0	0	
hormone)	1	1.6	4.69	291.6 x 10^6	0	0	0	
	2	1.7	5.59	326.64 x 10^6	0	0	0	

(20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6). It was concluded that tilapia fed solely on raw *Spirulina* could keep normal reproduction throughout three generations (Lu & Takeuchi, 2004).

Previous work on European seabass (*Dicentrarchus labrax*) showed that two injections of GnRHa induce final oocyte maturation and spawning. The use of GnRHa alone during the last stages of vitellogenesis is enough to induce final oocyte maturation in European sea bass. In this study, we show for the first time the effect of the LHRHa on final maturation compared with the negative control. The results showed that injected and implanted LHRHa resulted in a higher frequency of mature fish, eggs and sperms compared with the negative control. Mengumphan *et al.* (2006) reported that in ten years old Mekong giant catfish in earthen pond injected with LHRHa 50 ug/kg once a week indicated higher level of steroid and number of mature fish than control group after injection. The studies making the use of GnRha with two injections were the most efficient in inducing final oocyte maturation and spawning, higher oocyte diameter and the higher percentage of oocytes in germinal vesicle breakdowns at 48 h after the second injection (Francisco *et al.,* 2001).

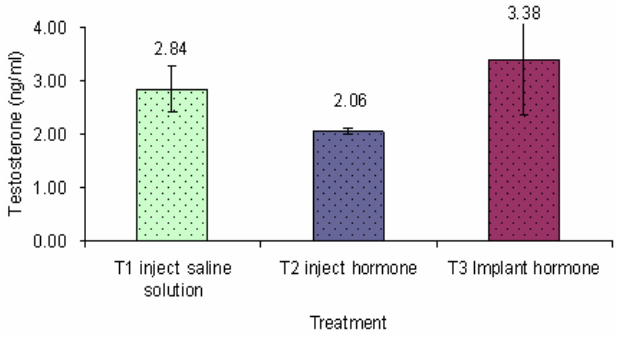
This study constitutes information for the establishment of seed production for this catfish in commercial hatcheries. We further recommend studies focused on micronutrient composition analysis such as vitamin and fatty acid both in pellet and eggs. The hybridization between two population should be considered for commercial production and breeding.

CONCLUSION

Based on results, it is concluded that supplementing SP in feeds of *P. bocourti* may have great benefits in the reproduction. Intramuscular administrations of LHRHa were effective for the artificial propagation of *P. bocourti* in the spawning season.

Fig. 6. Gain weight (g) of *P. bocourti* (north population) during April–June 2008 as a response to LHRHa hormone

Fig 7. Testosterone (ng/mL) of *P. bocourti* (north population) as a response to LHRHa hormone in July 2008



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