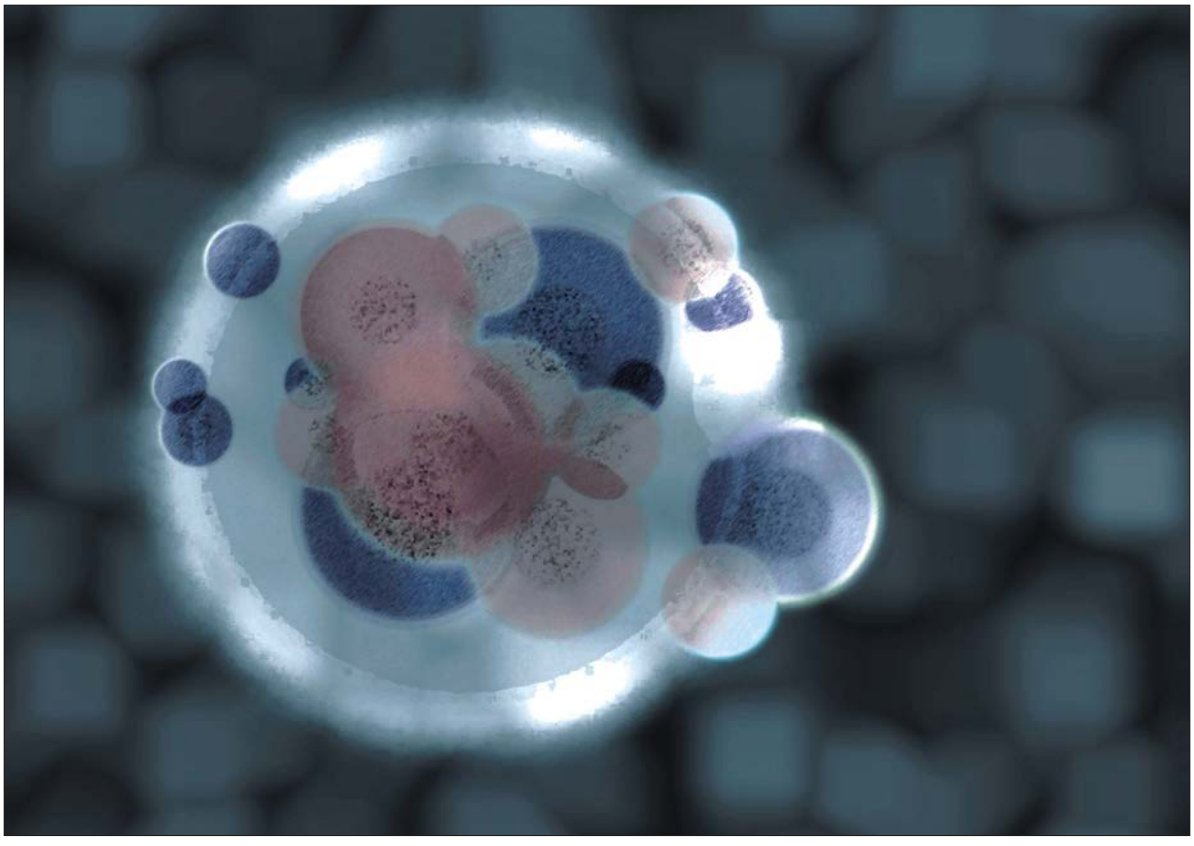

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.289.296

Research Article
Effects of Melatonin and Zinc Amino Acid on Female Walking Catfish (*Clarias macrocephalus*) Broodstock Performance

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Abstract

Background and Objective: *Clarias macrocephalus* is an important aquaculture species and have generated much interest in artificial propagation, especially in enhancing the broodstock performance and larval quality for aquaculture production. This study examined the broodstock performance effects of the combined zinc amino acid and melatonin treatment to the female walking catfish, *Clarias macrocephalus*. **Materials and Methods:** The varied zinc amino acid levels and melatonin of Control (0 ppm zinc amino acid and melatonin), MZn1 (100 ppm zinc amino acid and 50 mg kg^{-1} melatonin) and MZn2 (200 ppm zinc amino acid and 50 mg kg^{-1} melatonin) were mixed in the diet comprised of isonitrogenous and isocaloric of 37% crude protein and 9.3% crude lipid and were fed to the female catfish. Zinc accumulation, maturation analysis, breeding performance and immune analysis were evaluated. **Results:** The results of this study revealed that the combined zinc amino acid and melatonin treatment (MZn1 and MZn2) significantly increased the maturation parameters and reproductive performance after 8 weeks of treatment. There was also a significant increase in haematological parameters within the combined zinc amino acid and melatonin treatment such as red blood cell count and haematocrit. **Conclusion:** This study recommended the combined zinc amino acid and melatonin treatment of the dosage of 200 ppm zinc amino acid and 50 mg kg^{-1} melatonin (MZn2) to enhance the female maturation of *C. macrocephalus*.

Key words: Melatonin, zinc amino acid, maturation parameters, breeding performance, female walking catfish

Received: March 22, 2018 Accepted: May 25, 2018 Published: July 15, 2018

Citation: Siti-Ariza Aripin, Orapint Jintasataporn and Ruangvit Yoonpundh, 2018. Effects of melatonin and zinc amino acid on female walking catfish (*Clarias macrocephalus*) broodstock performance. J. Biol. Sci., 18: 289-296.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Clarias macrocephalus or walking catfish is widely distributed in Asia. In Thailand, *C. macrocephalus* has been commercially cultured due to its tender flesh and delectable flavour[1,2]. Although the locals prefer *C. macrocephalus*, its production is limited due to the introduction of catfish hybrids and decreasing habitat due to urbanization and over-exploitation[3]. Additionally, the development of aquaculture farming of *C. macrocephalus* has resulted in high demand of *C. macrocephalus* broodstock. However, *C. macrocephalus* broodstock has become limited due to overexploitation of the natural habitat. According to Barrero *et al.*[4], the timing of sexual maturity and successful spawning programme are vital to operate the commercial catfish farming. Several programmes have been developed to accelerate the sexual maturation in cultured catfish including hormonal and environmental manipulations[4,5].

According to Bayarri *et al.*[6], photoperiod is one of the important environmental factors that regulate the reproductive cycle. Previous studies have shown that manipulating the photoperiod may affect the sexual maturation[6,7]. Melatonin is an indolamine hormone synthesized in the pineal gland and is controlled by the photoperiod. During the night cycle, two enzymatic steps allow the formation of serotonin from tryptophan[8]. Tryptophan hydroxylation catalysed by tryptophan hydroxylase (TpOH) allows the synthesis of hydroxytryptophan. The hydroxytryptophan is then decarboxylated by the aromatic amino acid decarboxylase, leading to the formation of serotonin. Next, the arylalkylamine N-acetyltransferase (AANAT) catalyses the formation of N-acetylserotonin and the hydroxyindole-O-methyltransferase (HIOMT) converts the N-acetylserotonin into melatonin[8,9]. Amano *et al.*[10] has suggested that melatonin was involved in the reproductive processes through the hypothalamus-pituitary-gonadal endocrine axis. Previous *in-vitro* studies reported that melatonin induction into the preoptic anterior hypothalamic area (POAH) and pituitary stimulated the luteinizing hormone (GTH-II) levels[11]. Additionally, Amano *et al.*[10] stated that melatonin might also stimulate GTH-I by activating the sGnRH synthesis in the brain. Besides stimulating GTH-I and GTH-II, melatonin has direct effects in ovarian functions. Melatonin receptors are found in the ovary, brain and other peripheral tissues. According to Cutando *et al.*[12], the direct effect of melatonin receptor mechanism is by its metabolites, functioning as direct scavengers of free radicals and an indirect antioxidant. Zinc is an essential trace element required during developmental processes, being cofactor for enzyme activities such as DNA and RNA polymerases[13]. According to Menezo *et al.*[14], zinc is one of the most abundant transition metals, it binds strongly to metalloproteins (metallothionein/cystine-rich proteins) in the liver. Metallothionein stabilizes DNA conformation through its involvement in numerous DNA repair enzymes, especially during early embryogenesis[14,15]. Vitellogenin for oocytes that form the major yolk proteins for the developing embryo and larvae, formed in the hepatic cells has an abundant of metallothionein[16]. Zinc deficiency may lead to growth impairment and congenital malformation of foetal organs[17].

In recent years, melatonin and zinc amino acid in separate treatment showed significant improvement towards the maturation of female and male *C. macrocephalus*[18,19]. To further the understanding of the influence of melatonin and ZnAA in female *C. macrocephalus*, this research investigated the optimum level of combined melatonin and zinc amino acid treatment to enhance the female *C. macrocephalus* broodstock maturation and reproductive performance.

MATERIALS AND METHODS

Animals: The *Clarias macrocephalus* female broodstock were obtained from the Fisheries Station of Kham Pheng Phet, Department of Fisheries, Ministry of Agriculture and Cooperative, Thailand and the trial experiments were done in the Laboratory of Nutrition and Aquafeed, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. The 8 weeks old catfish were acclimatized in 500 L tanks at the density of 15ind/m^2/fish/tank and fed with control feed for 2 weeks prior to the experiment. The animal experiment was done in compliance with the Ethical Principles and Guidelines for the Use of Animals of the National Research Council of Thailand. The source of water supply for the experiment was aerated to maintain the oxygen supply in the experiment tank.

Experimental diets: Feeding trial was performed using one control and two combined zinc amino acid and melatonin diet. The basal diet was formulated from practical ingredients containing 22% fishmeal, 35% soybean, 1% spirulina, 12% wheat flour, 11.8% tapioca, 5% ricebran, 2% fish oil, 3% soy oil, 1.2% mineral premix, 2% soy lecithin, 1.5% calcium phosphate, 1% attractant, 2% binder and 0.5% vitamin premix. The diet also consisted of 37% crude protein and 9.3% crude lipid. Diets containing combined zinc amino acid and melatonin were prepared by adding graded levels of zinc amino acid and

melatonin to the basal diet. The combined zinc amino acid (ZnAA) and melatonin concentrations in the diet were 0 (control), 100 ppm ZnAA and 50 mg kg^{-1} melatonin (MZn1) and 200 ppm ZnAA and 50 mg kg^{-1} melatonin (MZn2)[20,21].

Experimental condition: A total of 45 females of mean weight 63.85±4.97 g (Mean±SD) were starved in the tanks for 2 days prior to the experiment. All experiment population was subjected to normal photoperiod (12 h day light) prior to the treatment. The fish were fed at a level equivalent to 3% of their body weight and this amount of diet was divided into two equal feedings per day. The fish were randomly distributed in three treatments (Control, MZn1 and MZn2) and with three replicates. Following acclimation, the fish were exposed to the treatments for 8 weeks.

Zinc analysis: The zinc analysis was done using inductively coupled plasma optical emission spectrophotometer (ICP-OES) in Central Laboratory (Thailand) Company Limited. An amount of 0.25-2 g of samples was prepared for the analysis. The prepared samples were injected and analyzed inan ICP-OES machine. The results were compared with the standard curve to determine the zinc concentration[22].

Growth performance: Female broodstock was weighed before final sampling to determine the growth performance by using the following formula:

$$\text{Weight gain: WG (\%)} = \frac{\text{Final body weight-Initial body weight}}{\text{Initial body weight}} \times 100$$

Histology: The ovaries from each treatment were fixed in 10% buffered formalin. Serial sections were made at four micrometer and were processed using the standard method of histology for sample cutting. For staining method, the samples were stained using haematoxylin-eosin method. The histology method was according to Drury and Wallington[23]. The percentages of peri-nucleolus stage and TYS cells were counted under a microscope.

Gonadosomatic index: The Gonadosomatic index was determined as[24]:

$$GSI = \frac{Gm}{Tm} \times 100$$

Where:

Gm = Mass of Gonad

Tm = Total mass of fish

Hematological parameter: At the end of the 8 weeks trial, the total red blood cells and white blood cells were counted using an improved Neuberhaemacytometer. The blood was diluted at 1:200 with Dacie and Lewis[25] solution and was counted in the loaded haemacytometer chamber under the light microscope[25]. The estimation of haemoglobin was done following Drabkin and Austin[26] where well-mixed anticoagulated blood was diluted in Drabkin's solution and the haemoglobin was measured at 540 nm. Finally, hematocrit assay was done using hematocrit capillary tubes spun in Gemmy model KHT-410E Hematocrit Centrifuge and was measured in duplicate.

Immune analysis: The immunoglubolin-M method followed the standard method by Lowry *et al.*[27] where the serum was pre-treated with copper ion in alkaline solution. The phosphomolybdate phosphotungstic in the treated samples were reduced by the Folin reagent. The analysis was estimated using spectrophotometer (750nm) against a standard bovine serum albumin solution.

Estradiol analysis: The serum from the treatments was used for estradiol analysis. The procedure followed the IMMULITE® Estradiol by Siemens Medical Solution Diagnostic. The estradiol-enzymes conjugate competed with serum samples, the chemiluminescent substrate was added and the signal was generated according to the bound enzyme.

Artificial breeding: After 8 weeks of treatment, artificial breeding was done with the remaining female *C. macrocephalus* from each treatment to assess the reproductive performance. Prior to breeding assessment, the broodstocks were anaesthetized, using clove oil. The female *C. macrocephalus* were weighed prior to the breeding session. A mixture of 30 µg buserelin acetate (LHRH analogue) and 10 mg domperidone (dopamine analogue) was used to induce 1 kg female broodstock (0.1 mL mixture for 100 g females). The stripping was done after 16 h of the artificial induction. Male African catfish semen was used as the control male for the artificial spawning in order to standardize the male semen. The semen was diluted in normal saline water and separated for every replicates. The eggs and semen were added to the same bowl for fertilization and the fertilized eggs were then transferred to the hatching tanks for incubation. Egg production, fertilization rate, hatching rate, gonadosomatic index (GSI) and survival rate (seven days old) of the larvae were investigated after the artificial spawning session[28].

Fish reproductive characteristics: After the breeding session, egg production, egg quality and larval quality were determined. Egg production was estimated by directly counting spawnable eggs in the female ovaries[29]. Thirty eggs from each sample were measured using Motic microscope (Motic BA210 Digital Laboratory Microscope with Moticam 1000 camera) for egg diameter measurement.

Statistical analysis: All data were analyzed by one-way ANOVA (analysis of variance), followed by the Tukey's honest significance test to analyze the significance between the treatment means where the significance of all means comparisons was tested at $p<0.05$ using a SPSS software[30].

RESULTS

The *C. macrocephalus* female broodstock were subjected to zinc amino acid accumulation analysis after 8 weeks of experiment trial. The zinc amino acid concentrations in serum, meat and liver were significantly different between the treatments with the mean ranging from 6.55-8.90 mg kg^{-1} wet weight ($p = 0.043$), 6.97-9.14 mg kg^{-1} wet weight ($p = 0.019$) and 20.82-28.70 mg kg^{-1} wet weight ($p = 0.002$), respectively (Table 1). However, there was no statistical difference for bone and ovary with the mean ranging from 45.31-49.10 mg kg^{-1} wet weight ($p = 0.1$) and 42.75-44.83 mg kg^{-1} wet weight ($p = 0.1$), respectively (Table 1).

Combined dietary zinc and melatonin treatment had no significant effect on the weight gain with the mean weight gain ranging from 8.7-9.5 % ($p = 0.8$). Similarly, estradiol profile after 8 weeks of combined zinc and melatonin treatment was not significantly different with the mean ranging from 3049.5-5081.0 pg mL^{-1} ($p = 0.2$) (Table 2). For histological analysis, most ovaries in the control group contained the highest percentage of peri-nucleolus stage (PNS) with the percentage of 45% (control), 35.2% (MZn1) and 34% (MZn2) (Fig. 1, Table 2). The ovarian histology for combined zinc and melatonin treatments after 8 weeks was 37% (control), 50.9% (MZn1) and 54.7% (MZn2) for tertiary yolk stage (TYS) (Fig. 1, Table 2). After the experimental trial, there was a significant increase in the gonadosomatic index (GSI), fecundity and egg diameter in the presence of zinc and melatonin treatment with the mean ranging from 5.02-7.45% ($p = 0.001$), 3743.5-7060.9 eggs/g ($p = 0.001$) and 1587.8-1676.0 µm ($p = 0.001$), respectively (Table 2).

After 8 weeks of combined zinc and melatonin treatment, the female brood stock were subjected to blood collection for hematological and immunoglobulin-M (IgM) analysis. It was found that there was a significant increase for red blood cell (RBC) count and hematocrit with the mean ranging from 0.92-1.17 Mcell mL^{-1} ($p = 0.004$) and 12.5-16.25% ($p = 0.022$), respectively (Table 3). However, there was no statistical difference for white blood cell (WBC) count and hemoglobin (Hb) with the mean ranging from 185 047-265 482 cell mL^{-1}

Table 1: Zinc concentration in serum, meat, liver, bone, egg and ovary with melatonin and different zinc levels

Treatments	Control	MZn1	MZn2	p-value
Serum (mg kg^{-1} wet weight)	6.63±1.2[a]	6.55±3.1[a]	8.90±0.5[b]	0.043
Meat (mg kg^{-1} wet weight)	9.14±1.4[a]	8.26±1.5[a]	6.97±1.1[b]	0.019
Liver (mg kg^{-1} wet weight)	20.88±3.0[b]	20.82±1.9[b]	28.70±6.5[a]	0.002
Bone (mg kg^{-1} wet weight)	48.68±2.7	45.31±2.6	49.10±6.1	0.100
Ovary (mg kg^{-1} wet weight)	42.75±2.5	44.83±0.8	43.20±2.2	0.100

[a,b]Values with different superscripts in a row differ significantly ($p<0.05$)

Table 2: Maturation analysis of female *C. macrocephalus* with melatonin and different zinc levels

Treatments	Control	MZn1	MZn2	p-value
Tertiary yolk stage (TYS)	37.00%	50.90%	54.70%	-
Peri-nucleolus stage (PNS)	45.00%	35.20%	34.00%	-
Gonadosomatic index (%) (S.E.)	5.02±0.8[b]	7.05±1.4[a]	7.45±1.8[a]	0.001
Fecundity (egg/g) (S.E.)	3743.50±405[b]	6235.90±1206[a]	7060.90±1152[a]	0.001
Egg diameter (µm) (S.E.)	1587.80±127[b]	1661.30±94[a]	1676.00±60[a]	0.001
Weight gain (%) (S.E.)	8.70±4.6	9.50±4.4	9.10±4.8	0.800
Estradiol (pg mL^{-1}) (S.E.)	3049.50±859	3100.50±578	5081.00±2462	0.200

[a,b]Values with different superscripts in a row differ significantly ($p<0.05$)

Table 3: Immune analysis of female *C. macrocephalus* with melatonin and different zinc levels

Treatments	Control	MZn1	MZn2	p-value
Red blood cell (Mcell mL^{-1}) (S.E.)	0.92±0.07[b]	1.15±0.06[a]	1.17±0.10[a]	0.004
Hematocrit (%) (S.E.)	12.50±1.29[b]	15.25±1.29[a]	16.25±2.22[a]	0.022
Hemoglobin (g dL^{-1}) (S.E.)	5.00±0.27	4.08±0.49	4.98±0.87	0.090
White blood cell (cell mL^{-1}) (S.E.)	265482.00±51222	185047.00±32550	216327.00±55707	0.100
Immunoglobulin M (mg dL^{-1}) (S.E.)	0.143±0.03	0.18±0.02	0.17±0.01	0.055

[a,b]Values with different superscripts in a row differ significantly ($p<0.05$)

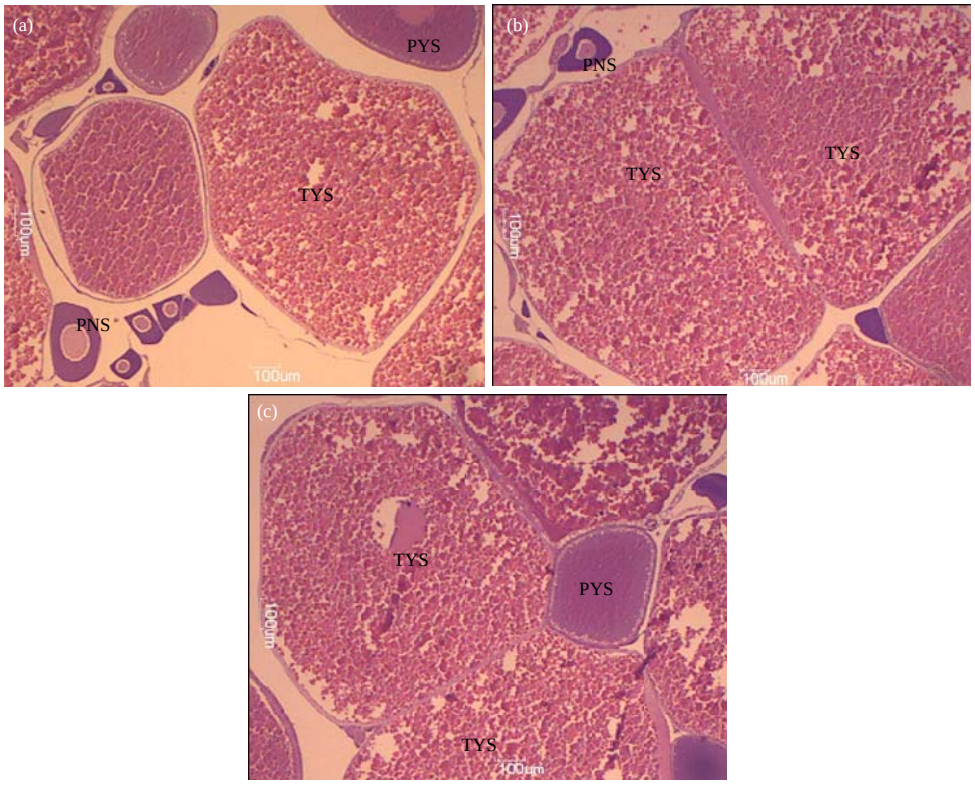

Fig. 1(a-c): Effect of melatonin and zinc treatment on ovarian histology of the *C. macrocephalus*. Cross section of an ovary of fish treated with (A) Control, (b) MZn1 and (c) MZn2. PNS: Peri-nucleolus stage, PYS: Primary yolk stage, TYS: Tertiary yolk stage
Scale bar = 100 µm

Table 4: Breeding performance of female *C. macrocephalus* with melatonin and different zinc levels

Treatments	Control	MZn1	MZn2	p-value
Egg production (egg/g) (S.E.)	3506.70±2770[b]	4082.00±3001[a]	6553.00±1515[a]	0.027
Fertilization rate (%) (S.E.)	24.60±18.9[b]	53.40±17.5[a]	54.90±21.2[a]	0.002
Survival rate (%) mean (S.E.)	28.33±20.0[b]	45.87±21.0[a]	52.49±22.0[a]	0.002
Final Gonadosomatic index (%) (S.E.)	0.52±0.2[b]	0.75±0.40[a]	1.77±1.70[a]	0.029
Hatching rate (%) (S.E.)	15.70±18	33.00±19.0	34.60±20	0.070

[a,b]Values with different superscripts in a row differ significantly (p<0.05)

(p = 0.1) and 4.08-5.0 g dL^{-1} (p = 0.09), respectively (Table 3). Similarly, no significant difference between the treatments were found in immunoglobulin-M assay (IgM) with the mean ranging from 0.143-0.18 mg dL^{-1} (p = 0.055) (Table 3).

The female broodstock were artificially spawned with pooled semen from control males to evaluate the reproductive performance after the 8 weeks of combined zinc amino acid and melatonin experiment trial. The egg production, fertilization rate, survival rate and final gonadosomatic index had significant differences after the combined zinc amino acid and melatonin treatment with the mean ranging from 3506.7-6553 egg/g (p = 0.027), 24.6-54.9% (p = 0.002), 28.33-52.49% (p = 0.002) and 0.52-1.77% (p = 0.029), respectively (Table 4). However, the zinc amino acid and melatonin treatment had no significant differences in the hatching rate with the mean ranging from 15.7-34.6% (p = 0.07).

DISCUSSION

After 8 weeks of combined zinc amino acid and melatonin feed treatment, the zinc concentration of serum, meat and liver were significantly different. Zinc absorbed from the digestive tract is transported via blood circulation to the target organs. According to Do Carmo e Sa *et al.*[31], normally the zinc in serum concentration is in homeostasis, the body will maintain the zinc concentration which only will be broken down in the extreme cases of very low or very high zinc intake.

The zinc concentration in the liver of *C. macrocephalus* was due to the accumulation of metallothionein. According to Do Carmo e Sa *et al.*[31], the zinc concentration which is stored in the liver are called metallothionein. Metallothionein may serve as a zinc donor during a high metabolic requirement for zinc. Vitellogenin from the liver derived from hepatic synthesis is abundant in metallothionein and is involved in oocytes growth prior to spawning. In the current study, the results suggested that a low zinc accumulation in the meat of fish treatment fed was due to the demand of zinc metabolism such as reproduction. The high demand might be due to the mix of melatonin and zinc feed that altered the zinc accumulation of the target organs due to the melatonin receptors. According to previous studies, melatonin receptors main structure consists of a DNA binding domain that contains a zinc double finger[32-34]. In this case, melatonin receptors might have altered the zinc metabolism in the treated female broodstock, reducing the zinc concentration in meat.

Gonadosomatic index (GSI), fecundity, egg diameter and histological analysis in the present study showed a significant difference in the combined melatonin and zinc amino acid feed compared to the control feed. The significant difference in maturation of gonad after melatonin treatment has been reported in previous studies such as rat, salmon, *Channa punctatus,* zebrafish and Japanese medaka[10,35-37]. According to Amano *et al.*[10], the melatonin treatment had a stimulatory effect on follicle stimulating hormone (GTH-I). Mazurais *et al.*[38] and Amano *et al.*[10], suggested that melatonin may affect the sGnRH synthesis in the brain and regulate GTH-I secretion in hypothalamic-pituitary-gonadal. The role of zinc in the current study confirmed the association between zinc and gonad maturation where zinc enhanced the GSI, fecundity, egg diameter and histological analysis. It was supported by previous study on important role of zinc in the synthesis and secretion of GTH-I and GTH-II mediated by the zinc fingers located in the nuclear receptors[39].

According to Srivastava and Choudhary[40], blood cell indices are good indicators of systemic response to external stimulus. The increase of red blood cell count and hematocrit concentration in the present study suggested that melatonin had the stimulatory effect of bone marrow or gone through the stimulation of some cytokines for bone marrow cell proliferation[41-43]. On the other hand, zinc enhances the immune system by regulating the physiological pathways in the bone marrow, which needs zinc for growth and maturation[44]. Do Carmo e Sa *et al.*[31] stated that zinc deficiency increased the erythrocyte fragility and mightlead to anaemia. Furthermore, zinc also acts as antioxidant to maintain the integrity of the erythrocyte membrane from the action of free radicals[31,45]. The combined diet of melatonin and zinc clearly showed significant effects on the hematological parameters rather than treated separately in the previous studies[18,19].

In the present study, the larval quality parameters were significantly higher in the combined zinc and melatonin treatment, indicating that zinc and melatonin treatment was suitable in enhancing the broodstock performance of catfish. Previous study by Lombardo *et al.*[46] found similar results where melatonin administration was able to increase the number of spawned eggs. Lombardo *et al.*[46] and Tamura *et al.*[47] suggested that melatonin administration might enhance the spawned eggs by regulating the steroidogenic enzyme activities on gonadal steroidogenesis or by increasing melatonin gene expression in theca and granulosa cells in mammals. Zinc, on the other hand, enhances the embryo development by regulating the completion of meiosis and egg activation[48,49]. Therefore, zinc from the feeding trial in the current study might provide a source of zinc for oocyte maturation and for the developmental stages following fertilization. Although this study provides important insights into the benefits of combined melatonin and zinc amino acid, this study may lack some understanding of mechanism between melatonin and zinc amino acid in reproductive performance of *C. macrocephalus* female broodstock. Thus, the present study provided a preliminary platform to further understanding on the mechanisms of zinc amino acid and melatonin on maturation and reproduction performance.

CONCLUSION

The combined actions of zinc amino acid and melatonin diet had beneficial effects in enhancing the maturation of female *C. macrocephalus*. This study recommended the combined zinc amino acidand melatonin treatment of dosage of 200 ppm zinc amino acid and 50 mg kg^{-1} melatonin (MZn2) to enhance the female maturation of *C. macrocephalus*.

SIGNIFICANCE STATEMENT

This study discovers that combined melatonin and zinc amino acid in the feed were able to enhance the maturation and reproductive performance of female *Clarias macrocephalus* broodstock with improved larval qualities. This study helps the researchers to cover the mechanism of melatonin and zinc amino acid working together in reproductive physiology of this particular species. Thus, a new theory on role of melatonin and zinc amino acid on enhancing the broodstock quality may be arrived at.

ACKNOWLEDGMENT

The authors are grateful to all members of Laboratory of Nutrition and Aquafeed, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok for their kind assistance during this study. The authors would like to thank the Ministry of Higher Education, Malaysia that had funded this study through scholarship bench fees (Reference No: KPT (BS) 840409085436).

REFERENCES

1. Petkam, R. and G.E.E. Moodie, 2000. Food particle size, feeding frequency and the use of prepared food to culture larval walking catfish *Clarias macrocephalus*. Aquaculture, 194: 349-362.
2. Areerat, S., 1987. *Clarias* culture in Thailand. Aquaculture, 63: 355-362.
3. Na-Nakorn, U., W. Kamonrat and T. Ngamsiri, 2004. Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. Aquaculture, 240: 145-163.
4. Barrero, M., B.C. Small, L.R. D'Abramo, L.A. Hanson and A.M. Kelly, 2007. Comparison of estradiol, testosterone, vitellogenin and cathepsin profiles among young adult channel catfish (*Ictalurus punctatus*) females from four selectively bred strains. Aquaculture, 264: 390-397.
5. Kestemont, P., J. Cooremans, A. Abi-Ayad and C. Melard, 1999. Cathepsin L in eggs and larvae of perch *Perca fluviatilis*: Variations with developmental stage and spawning period. Fish Physiol. Biochem., 21: 59-64.
6. Bayarri, M.J., L. Rodriguez, S. Zanuy, J.A. Madrid and F.J. Sanchez-Vazquez *et al*., 2004. Effect of photoperiod manipulation on the daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrarchus labrax*). General Comparat. Endocrinol., 136: 72-81.
7. Endal, H.P., G.L. Taranger, S.O. Stefansson and T. Hansen, 2000. Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. Aquaculture, 191: 337-349.
8. Falcon, J., H. Migaud, J.A. Munoz-Cueto and M. Carrillo, 2010. Current knowledge on the melatonin system in teleost fish. General Comparat. Endocrinol., 165: 469-482.
9. Falcon, J., 1999. Cellular circadian clocks in the pineal. Prog. Neurobiol., 58: 121-162.
10. Amano, M., M. Iigo, K. Ikuta, S. Kitamura, H. Yamada and K. Yamamori, 2000. Roles of melatonin in gonadal maturation of underyearling precocious male masu salmon. General Comparat. Endocrinol., 120: 190-197.
11. Khan, I.A. and P. Thomas, 1996. Melatonin influences gonadotropin II secretion in the Atlantic croaker (*Micropogonias undulates*). General Comparat. Endocrinol., 104: 231-242.
12. Cutando, A., J. Aneiros-Fernandez, A. Lopez-Valverde, S. Arias-Santiago, J. Aneiros-Cachaza and R.J. Reiter, 2011. A new perspective in oral health: Potential importance and actions of melatonin receptors MT1, MT2, MT3 and RZR/ROR in the oral cavity. Arch. Oral Biol., 56: 944-950.
13. Hayashi, K., H. Hara, P. Asvarujanon, Y. Aoyama and P. Luangpituksa, 2001. Ingestion of insoluble dietary fibre increased zinc and iron absorption and restored growth rate and zinc absorption suppressed by dietary phytate in rats. Br. J. Nutr., 86: 443-451.
14. Menezo, Y., L. Pluntz, J. Chouteau, T. Gurgan, A. Demirol, A. Dalleac and M. Benkhalifa, 2011. Zinc concentrations in serum and follicular fluid during ovarian stimulation and expression of Zn^{2+} transporters in human oocytes and cumulus cells. Reprod. Biomed. Online, 22: 647-652.
15. Jaroudi, S., G. Kakourou, S. Cawood, A. Doshi and D.M. Ranieri *et al*., 2009. Expression profiling of DNA repair genes in human oocytes and blastocysts using microarrays. Hum. Reprod., 24: 2649-2655.
16. Banks, S.D., P. Thomas and K.N. Baer, 1999. Seasonal variations in hepatic and ovarian zinc concentrations during the annual reproductive cycle in female channel catfish (*Ictalurus punctatus*). Comparat. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol., 124: 65-72.
17. Riggio, M., S. Filosa, E. Parisi and R. Scudiero, 2003. Changes in zinc, copper and metallothionein contents during oocyte growth and early development of the teleost *Danio rerio* (zebrafish). Comparat. Biochem. Physiol. Part C: Toxicol. Pharmacol., 135: 191-196.
18. Aripin, S.A., O. Jintasataporn and R. Yoonpundh, 2015. Effects of zinc amino acid in walking catfish (Clarias macrocephalus) female broodstock first sexual maturation. J. Aquacult. Res. Dev.,J. Aquacult. Res. Dev., Vol. 6. 6 10.4172/ 2155-9546.1000347.
19. Aripin, S.A., O. Jintasataporn and R. Yoonpundh, 2015. Effects of exogenous melatonin in *Clarias macrocephalus*male broodstock first puberty stage. J. Aquacult. Res. Dev., Vol. 6. 10.4172/2155-9546.1000307.
20. Clearwater, S.J., A.M. Farag and J.S. Meyer, 2002. Bioavailability and toxicity of dietborne copper and zinc to fish. Comparat. Biochem. Physiol. Part C: Toxicol. Pharmacol., 132: 269-313.
21. Rubio, V.C., F.J. Sanchez Vazquez and J.A. Madrid, 2004. Oral administration of melatonin reduces food intake and modifies macronutrient selection in European sea bass (*Dicentrarchus labrax*, L.). J. Pineal Res., 37: 42-47.

22. Altundag, H. and M. Tuzen, 2011. Comparison of dry, wet and microwave digestion methods for the multi element determination in some dried fruit samples by ICP-OES. Food Chem. Toxicol., 49: 2800-2807.
23. Drury, R.A.B. and E.A. Wallington, 1967. Carleton's Histological Technique. 4th Edn., Oxford University Press, London, Pages: 432.
24. King, M., 1995. Fisheries Biology Assessment and Management. 1st Edn., Blackwell Science, Oxford, England, Pages: 341.
25. Dacie, J.V. and S.M. Lewis, 2001. Practical Haematology. 9th Edn., Churchill Livingstone, London, UK., pp: 23-24.
26. Drabkin, D.L. and J.H. Austin, 1935. Spectrophotometric studies: II. Preparations from washed blood cells, nitric oxide hemoglobin and sulfhemoglobin. J. Biol. Chem., 112: 51-65.
27. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
28. Unuma, T., S. Kondo, H. Tanaka, H. Kagawa, K. Nomura and H. Ohta, 2004. Determination of the rates of fertilization, hatching and larval survival in the Japanese eel, *Anguilla japonica*, using tissue culture microplates. Aquaculture, 241: 345-356.
29. Bagenal, T.B., 1978. Aspects of Fish Fecundity. In: Ecology of Freshwater Fish Production, Gerking, S.D. (Ed.). Wiley-Blackwell Scientific Publications, New York, pp: 75-101.
30. Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedurs in Agricultural Research. 2nd Edn., John Wiley and Sons, New York, USA., Page: 680.
31. Do Carmo e Sa, M.V., L.E. Pezzato, M.M.B.F. Lima and P.M. Padilha, 2004. Optimum zinc supplementation level in Nile tilapia *Oreochromis niloticus* juveniles diets. Aquaculture, 238: 385-401.
32. Jetten, A.M., 2009. Retinoid-related orphan receptors (RORs): Critical roles in development, immunity, circadian rhythm and cellular metabolism. Nuclear Recept. Signal., Vol. 7. 10.1621/nrs.07003.
33. Smirnov, A.N., 2001. Nuclear melatonin receptors. Biochemistry (Moscow), 66: 19-26.
34. Slominski, R.M., R.J. Reiter, N. Schlabritz-Loutsevitch, R.S. Ostrom and A.T. Slominski, 2012. Melatonin membrane receptors in peripheral tissues: Distribution and functions. Mol. Cell. Endocrinol., 351: 152-166.
35. Renuka, K. and B.N. Joshi, 2010. Melatonin-induced changes in ovarian function in the freshwater fish *Channa punctatus* (Bloch) held in long days and continuous light. General Comparat. Endocrinol., 165: 42-46.
36. Carnevali, O., G. Gioacchini, C.C. Piccinetti, F. Maradonna, F. Lombardo, E. Giorgini and G. Tosi, 2010. Melatonin control of oogenesis and metabolic resources in Zebrafish. J. Applied Ichthyol., 26: 826-830.
37. Kowalska, A., R.K. Kowalski and Z. Zakes, 2011. The effect of selective cyclooxygenase (COX) inhibitors on Japanese medaka (*Oryzias latipes*) reproduction parameters. World Acad. Sci. Eng. Technol., 5: 261-265.
38. Mazurais, D., I. Brierley, I. Anglade, J. Drew and C. Randall *et al*., 1999. Central melatonin receptors in the rainbow trout: Comparative distribution of ligand binding and gene expression. J. Comparat. Neurol., 409: 313-324.
39. Salgueiro, M.J., M. Zubillaga, A. Lysionek, M. Sarabia and R. Caro, 2000. Zinc as an essential micronutrient: A review. Nutr. Res., 20: 737-755.
40. Srivastava, S. and S.K. Choudhary, 2010. Effect of artificial photoperiod on the blood cell indices of the catfish, *Clarias batrachus*. J. Stress Physiol. Biochem., 6: 22-32.
41. Ahmed, H.H., G.S. Essawy, H.A. Salem and M.A. Abd El-Daim, 2011. Effect of melatonin on some hematological parameters and immune status of broiler chicks. J. Agric. Sci., 3: 243-254.
42. Anwar, M.M., H.A. Mahfouz and A.S. Sayed, 1998. Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs. Comparat. Biochem. Physiol. Part A: Mol. Integrat. Physiol., 119: 493-501.
43. Lissoni, P., S. Barni, G. Tancini, F. Rovelli, A. Ardizzoia, A. Conti and G.J.M. Maestroni, 1993. A study of the mechanisms involved in the immunostimulatory action of the pineal hormone in cancer patients. Oncology, 50: 399-402.
44. Gruber, K. and L. Rink, 2013. The Role of Zinc in Immunity and Inflammation. In: Diet, Immunity and Inflammation, Calder, P. And P. Yaqoob (Eds.)., Woodhead Publishing, UK., pp: 123-156.
45. O'Dell, B.L., J.D. Browning and P.G. Reeves, 1987. Zinc deficiency increases the osmotic fragility of rat erythrocytes. J. Nutr., 117: 1883-1889.
46. Lombardo, F., G. Gioacchini, A. Fabbrocini, M. Candelma, R. D'Adamo, E. Giorgini and O. Carnevali, 2014. Melatonin-mediated effects on killifish reproductive axis. Comparat. Biochem. Physiol. Part A: Mol. Integrat. Physiol., 172: 31-38.
47. Tamura, H., Y. Nakamura, A. Korkmaz, L.C. Manchester, D.X. Tan, N. Sugino and R.J. Reiter, 2009. Melatonin and the ovary: Physiological and pathophysiological implications. Fertil. Steril., 92: 328-343.
48. Tian, X. and F.J. Diaz, 2012. Zinc depletion causes multiple defects in ovarian function during the periovulatory period in mice. Endocrinology, 153: 873-886.
49. Tian, X. and F.J. Diaz, 2013. Acute dietary zinc deficiency before conception compromises oocyte epigenetic programming and disrupts embryonic development. Dev. Biol., 376: 51-61.