Global Protein Expression Profiling of Female Soro Brook Carp (Neolissochilus stracheyi) by Proteomics

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Abstract : Soro brook carp (*Neolissochilus stracheyi*) is a medium- to giant-size fish which belongs to *Cyprinidae* family. It is a freshwater fish that lives in waterfalls and mountains. Nowadays, the number of soro brook crap in nature is decreasing according to incorrect fishing methods. The use of agricultural chemicals that damages its ecosystem and other species of fish also affects its breeding and spawning season. This research focuses on studying the global protein expression profile of female soro brook carp that may affect fish's breeding season and egg development using proteomic technique. Total serum proteins from soro brook carp were collected from Nan Province and protein content was determined by bicinchoninic acid method. The concentrations of total proteins observed were between 2.82 and 20.92 mg/ml. The proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). It was revealed that the molecular weights of proteins in serum were in range 14.4 to 97.0 kDa. Then, the global proteins expression profiles were performed by 2-dimensional gel electrophoresis (2-DE) using 13 cm IPG strip pH 3-10 nonlinear. The majority protein spots were observed in p*I* between 5 and 8 and some samples showed protein spots with p*I* greater than 8. For further investigation, the total protein expression of female soro brook carp could be analyzed by ImageMaster™ Platinum V 2005 program to gain detailed information and a better understanding of the global protein expression during spawning and breeding season of soro brook crap.

Keywords : Soro brook crap, Proteomics, *Neolissochilus stracheyi*, 2-DE

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1. Introduction

Soro brook carp (*Neolissochilus stracheyi*) is one of the most important freshwater fish that inhabitants in the highland area of Nan Province. It is the *Cyprinidae* family which is also known as a source of high-protein. Currently, the population of soro brook crap in nature is decreasing according to incorrect fishing methods. The use of agricultural chemicals damage its ecosystem and other species of fish also affect to its breeding and spawning season. In recent years, there are some researchers have focused on study to find a proteins that affect the development of eggs in female fish. Due to the protein expression levels depend on development stages of the female eggs and changes season [1]. Lipophosphoglycoprotein commonly referred to as vitellogenin is an interested protein, which more important to develop egg of female fresh water fish in breeding season[2].More research about the factors and limitations of this fish species is needed, as there is insufficient information available.

Proteomic approach is a leading technology for the high-throughput analysis of proteins. Proteomic technologies, including 2-dimentionalgel electrophoresis (2D-PAGE) and mass spectrophotometry (MS) have provided opportunities for identifying interested proteins as to be protein markers. 2D-PAGE is effective in separating complex protein samples and in quantifying protein levels. Proteins of interest can then be identified by mass spectrophotometry (MS) and/or tandem mass spectrometry (MS/MS)[3]. Therefore, this research aims to study the global protein expression of female soro brook crap that may affect to fish's breeding season and egg development by proteomics.

2. Materials and Methods

Sample preparation

The blood samples of female soro brook carp (*Neolissochilus stracheyi*) in breeding season were collected from the Wa river basin, Borkluea district, Nan province. The blood samples were collected in a 1.5 mL microcentrifuge tube, incubated for 30 min at room temperature to allow clotting, and then centrifuged at 1000 g for 10 min for removing the clot. The supernatant serum samples were stored frozen at -80°C prior to analysis. The concentration of the total proteins in serum sample was assayed with a protein assay kit by using bovine serum albumin (BSA) as a standard.

SDS- Polyacrylamide Gel Electrophoresis (PAGE)

Serum proteins were resolved by SDS-PAGE according to Laemmli, 1970[4], using a 1.00 mm x 8 cm x 10 cm polyacrylamide slab gen consisting of 12.5% resolving gel (29:1, acrylamide, N,N′-bis-methylene acrylamide) and a 4% stacking gel. Fifteen microgram of serum proteins were loaded with a low-range molecular weight calibration kit (GE Healthcare). Proteins samples were separated by applying a constant voltage of 100 Volts for 1 h 30 min or *until bromophenol dye reaching* the bottom of the gel. After electrophoretic separation, gels were stained with Colloidal Coomassie Brilliant Blue G-250 and destained by using deionized water.

Two-Dimensional Polyacrylamide Gel Electrophoresis(2D-PAGE)

2-DE was performed with IPGphor system (GE Healthcare). Two hundred micrograms of proteins was used and added with 20uM DTT. The samples were then diluted into a lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 65 mM DTE, 5 mM tributylphosphine (TBP) and 1.5% IPG buffer (pH 3-10 NL). After incubate periodically for 1 h and centrifugation at 12000 ×*g* for 20 min, the sample solutions were applied onto IPG strips (Immobiline DryStrip, 13 cm, pH 3-10 NL, GE Healthcare). IPGphor IEF (GE Healthcare) was performed under the following condition: gel rehydration was carried out for 12 h at 50 V and run in the programmed setting for a total of 20,000 Vh. After IEF, the IPG strips were equilibrated in equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% v/v glycerol, 2% w/v DTT and a trace of bromophenol blue) for 15 min and then subsequently alkylated in the same equilibration buffer, but replacing DTT with 2.5% w/v iodoacetamide (IAA) for 15 min. The IPG strips were placed on top of the 12.5% polyacrylamide gel (13×13 cm) and covered with 1.0% agarose. The second-dimensional separation was carried out at 40 mA per gel at 15°C until the bromophenol blue dye front reached the bottom of the gel. At the end of each run, the 2-DE gels were stained with silver staining and scanned using a scanner.

Image Analysis

The stained gels were scanned with the Image scanner for protein spot detection. The protein spots were detected and quantified using the Image scanner, UTA-1120(GE Healthcare), following the manufacturer's image scanner protocol.

3. Results and Discussion

Serum protein sample preparation and protein concentrations from female *Neolissochilus stracheyi*

In this study, serum samples of twelve females *Neolissochilus stracheyi* were investigated the global protein expression during bleeding season. The body weight of fish between 150 and 480 g and body length ~ 25-34 cm were selected for this study (Table 1). The protein serum samples were lyophilized and kept at -80°C until used. Serum protein concentrations were determined by using BCA method. It was revealed that the protein concentrations of serum from female *Neolissochilus stracheyi* were ranged between 2.83 and 20.93 mg/mL (as shown in Table 1).

Table 1 Body weight and body length of female *Neolissochilus stracheyi*

Sample No.	Body weight (g)	Body length (cm)	Serum protein concentrations (mg/mL)
1	480	34.0	9.58
2	240	28.0	9.79
3	150	25.0	4.45
4	180	26.0	3.13
5	250	28.5	4.58
6	270	29.5	11.85
8	190	26.5	3.35
9	250	29.0	20.93
10	180	26.0	8.08
11	200	26.5	2.83
12	480	32.0	18.40

Protein expression analysis by SDS-PAGE

To investigate the serum protein expression of female *Neolissochilus stracheyi*, 12.5% SDS-PAGE was performed. The results were shown in Figure 1. It was revealed that the protein molecular weight between 14.4 and 97.0 kDa were observed in all serum protein samples. However, the expressions of proteins in each sample were slightly different. Proteins larger than 30 kDa were highly abundance in serum protein sample no. 1, 2, 3, 9 and 12. On the other hand, proteins with molecular weight ~ 15 kDa were highly expressed in serum protein sample no. 5. To gain insight the global protein expression, all serum protein samples were analyzed by 2-dimensional electrophoresis.

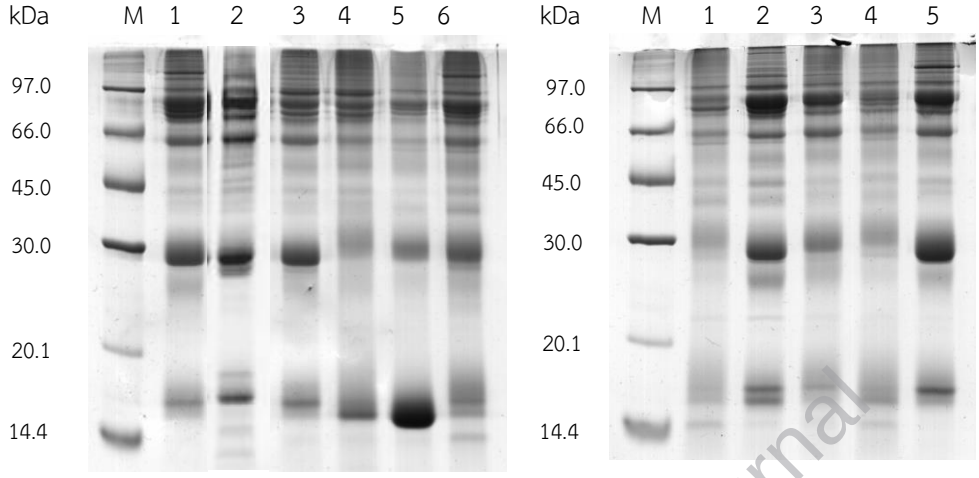
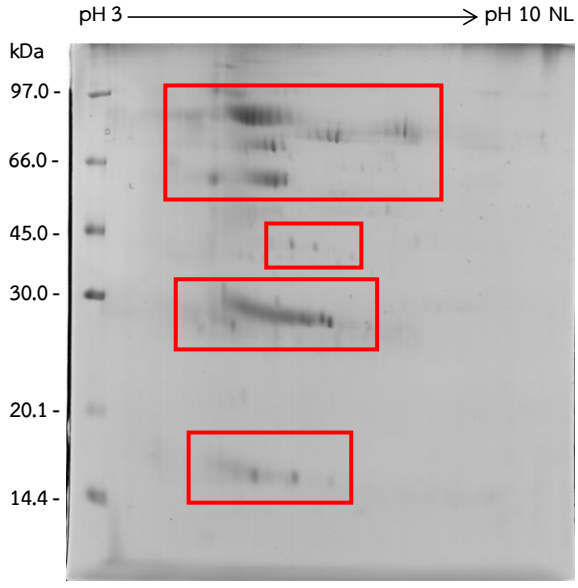


Figure 1 12.5% SDS-PAGE of15 μg total serum proteins from female *Neolissochilus stracheyi* (A) Lane 1-6 were protein sample number 1, 2, 3, 4, 5 and 6. (B) Lane 1-5 were protein sample number 8, 9, 10, 11 and 12, respectively. Lane M was low range molecular weight protein marker.

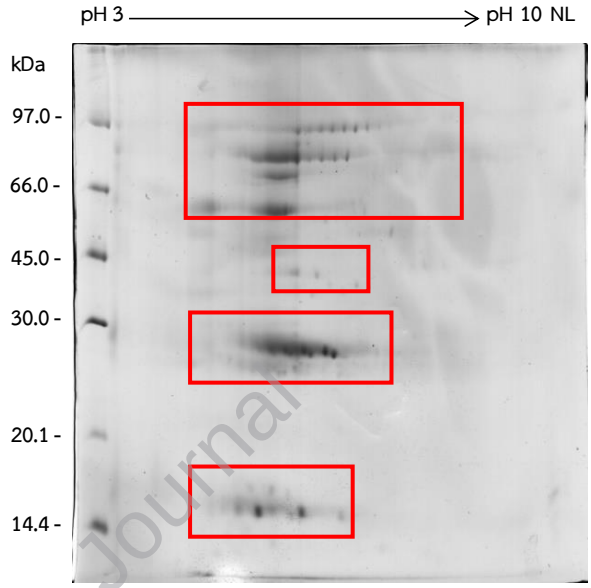
Global protein expression analysis by 2-DE

To investigate the global protein expression of serum proteins from female *Neolissochilus stracheyi*, 2-dimensional electrophoresis (2-DE) was performed in IPG strip 13 cm (pH 3-10 nonlinear, GE Healthcare). After stain gel with Colloidal Coomassie Brilliant Blue G-250 and silver staining, the results were shown in Figure 2. The pattern of protein expression in 2-DE were revealed slightly alkaline to mildly acidic, pH 5-8.The global protein expression of serum protein samples were slightly different. In sample no. 1 and 3, protein highly expressed in four different areas which were between 66.0-97.0 kDa at pH 5-8, 30.0 kDa, 44.0 kDa and 15 kDa at pH 5-7. It was obvious that two spots of proteins at molecular weight 44.0 kDa and 15.0 kDa were highly expressed.

In serum proteins no. 4 and 5, proteins at molecular weight between 66.0-97.0 kDa also expressed slightly in alkaline to mildly acidic, pH 5-8.However, proteins at molecular weight 15.0 kDa showed spots of proteins at pH > 8.0 and proteins spots with molecular weight less than 14.4 kDa were observed. In sample no. 6 and 8, spots of proteins were revealed alkaline to mildly acidic, pH 5-8. On the other hand, spots of proteins at pH > 8.0were not found. Proteins with molecular weight less than 14.4 kDa were also expressed. For serum proteins no. 9 and 10,alkaline to mildly acidic proteins, pH 5-8 also highly abundant. Mainly serum proteins were expressed at 66.0 -97.0 kDa, 30.0 kDa, 45.0 kDa and 15.0 kDa, pH 5-8. Proteins molecular weight 30.0 kDa of sample no. 9 and 10 showed highly expression than others samples and 15.0 kDa proteins were highly expressed at pH more than 8.0.



Sample No.1



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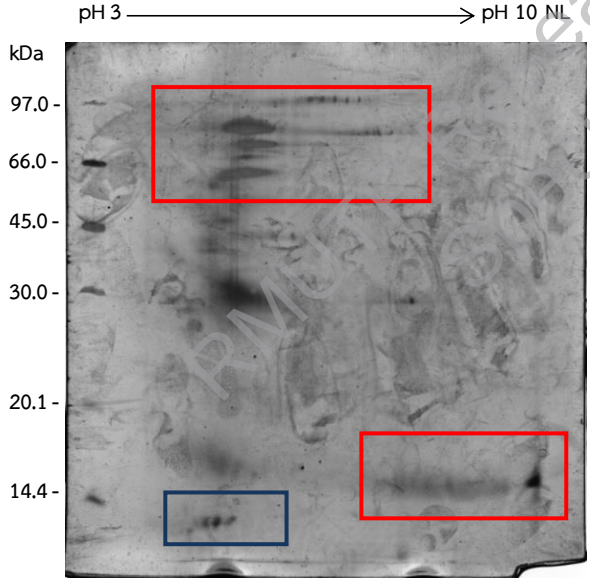
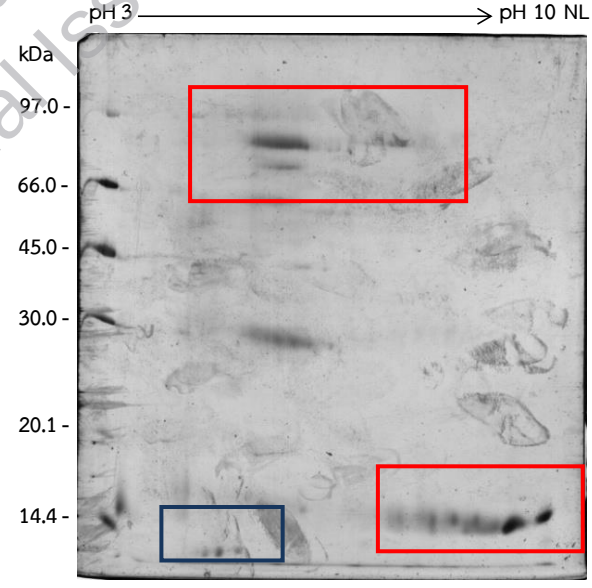


Sample No.4



Sample No.5

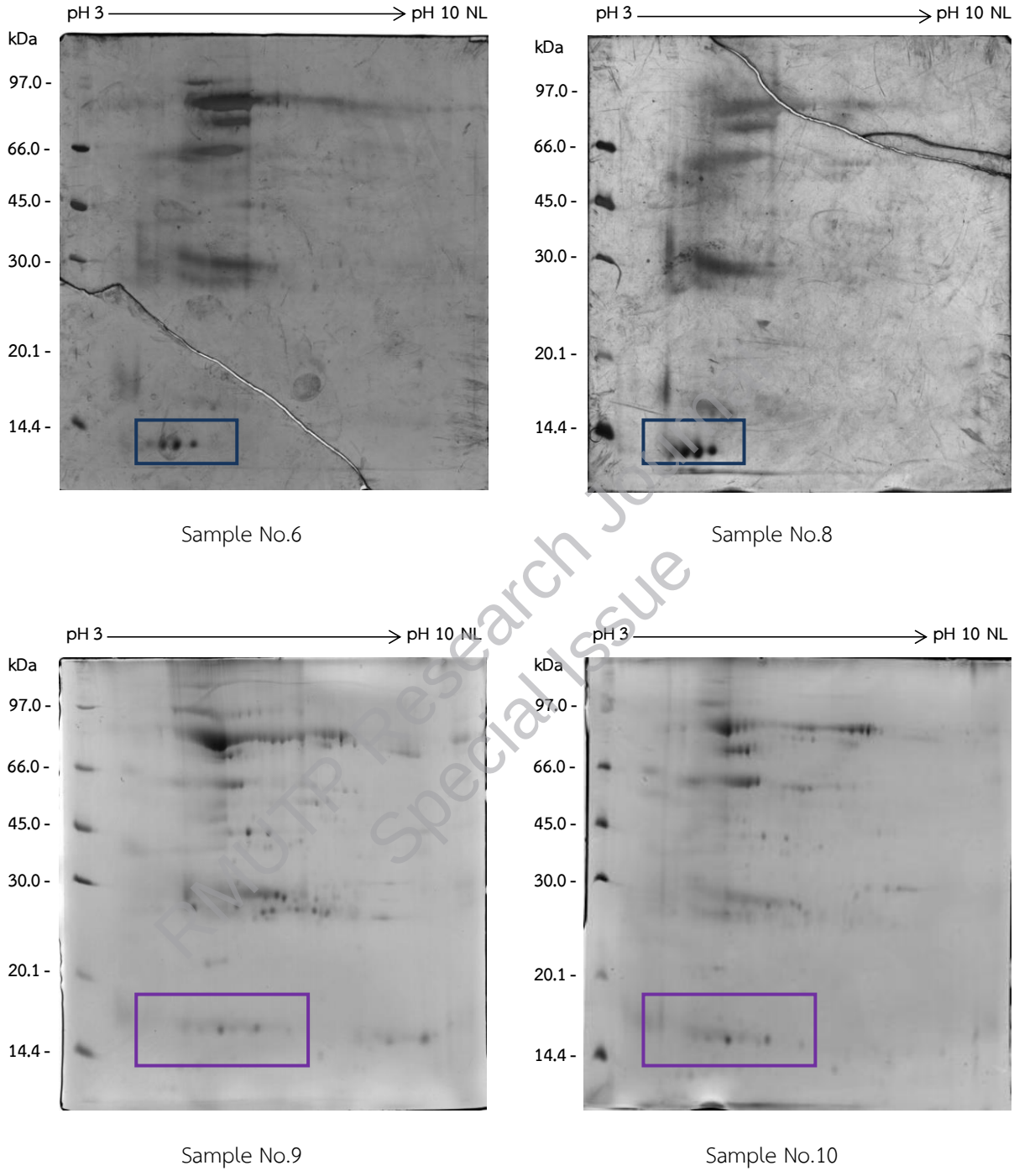


Figure 2 2-DE gels of protein expression of serum proteins from female *Neolissochilus stracheyi* sample number 1, 3, 4, 5, 6, 8, 9 and 10 after stained with Colloidal Coomassie Brilliant Blue G-250 and silver staining.

Oocyte maturation and yolk incorporation (vitellogenesis) is a hormonally controlled and regulated process. Prerequisite for successful fertilization in fish required oocyte maturation which occurred before ovulation. In many reports revealed three-step induction process which involved gonadotrophin (luteinizing hormone, LH and follicle stimulating hormone, FSH), maturation inducing hormone (MIH) and maturation-promoting factor (MPF) in fish models. LH was involved in the final maturation of gametes on the ovarian follicle layer to produce MIH (17α, 20β-dihydroxy-4-pregenen-3-one or 17α, 20β-BP) in most fish.[5]On the other hand, FSH has a dominant role in regulating vitellogenic growth of follicles, partly through stimulation of estradiol-17β (E2) biosynthesis by ovarian follicles. Moreover, E2 regulated ovarian development through the control of vitellogenin synthesis by hepatocytes in the liver during the oocyte growth period [6]. Vitellogenin is an important protein which was a female-specific serum protein and contained phosphorus, lipids, carbohydrates, calcium and iron. Vitellogenin has been identified as the egg-yolk precursor in most oviparous vertebrates.

Oogenesis was triggered by environmental and controlled by a series of regulating hormones as described before. For example, under the effect of temperature and/or photoperiod, the brain stimulated the pituitary gland to secrete gonadotropins [7]. A large number of researches has been directed at assessing the effects of organic molecules such as polycyclic aromatic hydrocarbons (PAHs) and nonylphenol polyethoxylate (NPEO) on different phases of vitellogenesis in fish and partly identified their mechanisms of action. PAHs have affected on the vitellogensis of fish from feral population as well as in laboratory experiments which would result in harmful sub-lethal effects with trans-generational consequences. This included the reduction in circulating hormones and plasma vitellogenin, estrogenic and antiestrogenic effects, retardation of oocyte maturation and reduction of reproductive success [8]. However, the lack of evidence of the changes of global protein expression during breeding season and effect of organic molecules on serum protein levels are still under investigated.

In many reports, FSH and LH have been studied and revealed their molecular weights around 32-33 kDa in many kind of fish [9-10]When the molecular weight of FSH and LH were compared with 2-DE gels of female *Neolissochilus stracheyi* in the experiment, it was found that serum protein expression also showed the protein spots around 30 kDa. For further investigation, the pattern of serum protein expression will be analyzed by using Image Master™ 2D Platinum version 5.0 to compare the different of protein spots in 2-DE gels. The spots of proteins which showed different of protein expression will be identified by mass spectrometry. However, it has not been reported about the global expression of protein of female *Neolissochilus stracheyi* between during and out of breeding season. This research may provide useful informative data for breeding development of *Neolissochilus stracheyi* for further investigation.

4. Acknowledgments

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