

Improvement of shrimp production sustainability and safety in Vietnam (BL/13/V06 – BL/12/V07)

Project partners (+indicate coordination):

University of Namur (FUNDP) : Prof. Patrick Kestemont (Belgian coordinator)

University of Liège (ULG) : Prof. Guy Maghuin-Rogister

Can Tho University (CTU) : Ass. Prof. Nguyen Thanh Phuong (Vietnamese coordinator)

Hanoi Agricultural University (HAU) : Prof. Dang Vu Binh

(Geographic) study area : Vietnam

Science policy testsite (if applicable) :

Project period: 2004-2006

Funding framework: Bilateral collaboration between Belgian Science Policy Office (BSPO) and Ministry of Science and Technology of Vietnam (MOST)

Data used (if satellite data, specify):

Context and objectives (max 14 lines)

Marine shrimp farming, mainly *Penaeus monodon*, has been put as a main target species for the development of the coming year plan in the Mekong Delta, Viet Nam. Improved extensive and semi-intensive farmings are the most common cultivation methods. The total shrimp culture area of the Mekong delta reached 498,234 ha in 2005 and the production attained 259,477 tons (78.6% of total national shrimp production). The rapid expansion of shrimp farming in the Mekong delta has led to a number of concerns, especially in terms of sustainable development such as **(i)** rapid expansion of shrimp farming, while the planning for sustainable and infrastructure development lags behind; **(ii)** increased use of chemicals and drugs (especially antibiotics and pesticides), causing extreme effects on the aquatic environment and on shrimp quality, including its physiological stress and health and residue contamination in final products; and **(iii)** conversion of mangrove forest area and rice production land to shrimp farming. The objective of the project was to evaluate the effects of Vietnamese shrimp production methods and the use of antibiotics on shrimp physiology and health as well as on the quality and safety of the final product in order to develop methodologies for the analysis of shrimp quality and safety, in respect with international food regulations.

Methodology Survey of chemical and drug use in shrimp farming in the North and South of Vietnam

- Shrimp, water and sediment samplings within selected farms and in the surrounding aquatic areas as well as in mangrove areas.
- Development and validation of analytical methods for selected antibiotic residues, including rapid screening by ELISA and bacteria-based test or simple chromatographical methods of detection and confirmation and quantification by liquid chromatography coupled to multiple mass spectrometry (LC-MSⁿ). Validation of the analytical methods according to the criteria described in the EU Commission Decision 2002/657/EC in the framework of the ISO 17025 norm.
- Kinetics of antibiotics contamination/decontamination in shrimps under laboratory and field conditions and development of a physiological and molecular bio-marker system.
- Training of Vietnamese scientists and technicians to advanced techniques used in ecotoxicology, food quality and safety analysis during extended stages in Belgium and short training course in Can Tho University
- Publication of manual based on quality insurance for the analysis of antibiotics in shrimps according to European legislation and ISO 17025 norm.

Results

- Results from surveys indicated that 155 kinds of drugs and chemicals are currently used in shrimp farming in North and South Viet Nam, including 31 antibiotics, 49 products used as pesticides and disinfectants, and 21 for water treatment. Fluoroquinolones (enrofloxacin, norfloxacin) are now frequently used by shrimp farmers.
- Rapid screening methods (ELISA and Belgian kidney test) have been adapted to shrimp tissues
- Very sensitive analytical methods based on LC-MSMS have been successfully developed for the quantification of residues of antibiotics and their validation. The research was focused on 3 classes of antibiotics: nitrofurans, (fluoro)quinolones and phenicols. Those developments and the validation of the nitrofurans method of analysis were done according to European legislation and ISO 17025 norm. The techniques have been applied to shrimp, water and sediment samples collected in laboratory experiments, shrimp farms and mangrove areas.

- Methodologies to measure oxidative stress biomarkers as well as AChE and ECOD activities have been successfully adapted in different shrimp tissues (hepatopancreas, gills and/or hemolymph). This set of biomarkers was used to assess the health status of shrimp exposed to 2 antibiotics (enrofloxacin or furazolidone) during contamination and decontamination phases in laboratory and in field conditions (intensive and improved extensive systems). Additionally, these biomarkers were measured in shrimps sampled in mangrove and organic farm areas. Moreover, experimental conditions have been defined to run 2 dimensional gel electrophoresis on shrimp hemolymph, and the "Protein Expression Signatures" have been obtained in shrimps exposed to antibiotics in laboratory or field conditions. It emerges from laboratory and field experiments that, even if significant residue accumulations occur in muscle (sometimes about the critical limit), furazolidone and enrofloxacin have only few effects on biochemical biomarkers (oxidative stress, AchE) and on protein expression profiles in *Penaeus monodon*. However, the farming system seems to significantly affect these biological parameters. The most sensitive biomarkers to be selected for further investigations are : LPO, GST, GPx, CAT, GSH, AChE and PES (protein expression signature).
- Four Vietnamese scientists have been trained to analytical (ELISA, Belgian kidney test, LC-MSMS) and biochemical (biomarker system) methods through extended stages in Belgium (ULG and FUNDP) and *in situ* training in Can Tho University to end-users (11 peoples, representative of governmental agencies for the control of fisheries products). A manual based on quality insurance for the analysis of antibiotics in shrimps according to European legislation and ISO 17025 norm will be edited soon.

Products and services (if applicable)

Website (with project results): www.ctu.edu.vn/aquaculture
Pictures illustrating the project: see annex 1

Discipline (select one or more appropriate disciplines)

Aquaculture
 Environment/nature conservation
 Food security/safety
 Agriculture Environment

Publications

Articles

- Douny, C., Widart, J., Scippo, M.L., De Pauw E., Silvestre, F., Kestemont, P., Maghuin-Rogister, G. 2006. Determination of four phenolic residues in shrimps by liquid chromatography-mass spectrometry. Food Additives and Contaminants (under revision)
- Douny, C., Widart, J., Focant, J.F., Scippo, M.L., Silvestre, F., Kestemont, P., De Pauw E., Maghuin-Rogister, G. 2006. An HPLC-MS/MS method for the monitoring of nitrofurans metabolites in shrimps" J. Chromatography B (submitted)
- Tu, H. T., Phuong, N.T., Silvestre F., Douny C., Tao, C.T., Maghuin-Rogister GR. and Kestemont P., 2006. Investigation on the use of drugs and chemicals in shrimp farming and the kinetics of Enrofloxacin and Furazolidone in black tiger shrimp (*Penaeus monodon*). Scientific Journal, Cantho University (In Vietnamese).
- Tu, H. T., Silvestre F., Douny C., Phuong, N.T., Tao, C.T., Guy Maghuin-Rogister GR. and Kestemont P., 2006. Residues analysis and oxidative stress response in black tiger shrimp (*Penaeus monodon*) exposed to enrofloxacin under laboratory and field conditions. In preparation (to be submitted to Aquatic Toxicology)
- Tu, H. T., Silvestre F., Douny C., Phuong, N.T., Tao, C.T., Maghuin-Rogister GR. and Kestemont P., 2006..Residues analysis and oxidative stress response in black tiger shrimp (*Penaeus monodon*) exposed to furazolidone under laboratory condition. In preparation (to be submitted to Ecotoxicology and Environmental Safety).
- Tu, H. T., Silvestre, F., Bernard A., Douny, C., Phuong, N.T., Tao, C.T., Maghuin-Rogister GR. and Kestemont P., 2006. AChE and ECOD activity in black tiger shrimp (*Penaeus monodon*) exposed to furazolidone or enrofloxacin. In preparation (to be submitted to Comparative Biochemistry and Physiology, part C)
- Silvestre, F., Tu, H. T., Douny, C., Phuong, N.T., Tao, C.T., Maghuin-Rogister GR. and Kestemont P., 2006. Differential in gel electrophoresis (DIGE) to identify biomarkers of exposition to antibiotics in the hemolymph of the giant tiger shrimp *Penaeus monodon* under different farming management systems in Vietnam. In preparation (to be submitted to Journal of Proteome Research).

Abstracts of conferences and posters

- Kestemont P., Silvestre F., Tu H.T., Douny C., Forget M.C., Phuong N.T., Maghuin-Rogister G., 2005. Effects of antibiotics on the contamination, physiology and health of tiger shrimp *Penaeus monodon*. Methodological approach and preliminary data. Abstracts of the World Aquaculture Society Conference, Bali, Nusa Dua, 9-13 May 2005, 452.
- Silvestre F., Tu, H.T., Douny, C., Maghuin-Rogister, G., Phuong, N.T., Kestemont, P., 2006. Proteomic analysis to identify biomarkers of exposition to antibiotics in the hemolymph of the giant tiger shrimp *Penaeus monodon*. Abstracts of the SETAC Europe 16th annual meeting, Controversies and solutions in Environmental Sciences. 7-11 May 2006, The Hague, Netherlands, 266.
- Tu, H. T., Bernard A., Silvestre F., Douny C., Phuong, N.T., Tao, C.T., Maghuin-Rogister GR. and Kestemont P., 2006. Biomarker evaluation in the black tiger shrimp (*Penaeus monodon*) after exposure to enrofloxacin and furazolidone. Abstracts of the SETAC Europe 16th annual meeting, Controversies and solutions in Environmental Sciences. 7-11 May 2006, The Hague, Netherlands, 265.
- Maghuin-Rogister, G., Douny, C., Widart, J., Silvestre, F., Kestemont, P. 2004. Improvement of shrimp production sustainability and safety in Vietnam. Wageningen (NL) (September 2004).
- Douny, C., Widart, J., Scippo, M.-L., Maghuin-Rogister, G., Silvestre, F., Kestemont, P. 2004. Improvement of shrimp production sustainability and safety in Vietnam. Abstracts of Bioforum, December 2004, Liège,
- Douny, C., Widart, J., Scippo, M.-L., Maghuin-Rogister, G., Silvestre, F., Kestemont, P. 2005. Improvement of shrimp production sustainability and safety in Vietnam. Third AOAC Europe/Eurachem Symposium: Legal Limits on the road to food safety. March 2005. Brussels.
- Scippo, M.-L., Degand, G., Dang P. K. , Tu, H. T., Douny, C., Silvestre, F., Kestemont, P., Widart, J., De Pauw, E., Maghuin-Rogister, G. 2006. Screening of Antibiotics in Shrimp using the Belgian Kidney Test. Abstract of the 5th International Symposium on Hormone and Veterinary Drug Residues Analysis. May 16-19, 2006, Antwerp, 206.