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INNOVATION IN ACTION: TURNING IDEAS INTO REALITY

Chapter 30

Vitellogenin Dipstick: Rapid Detection of Sexual Maturity in Catfish Species for Shariah-Compliant Aquaculture Innovation

Roshani Othman[1,3], Nur Akmal Suliman[1], Farrah Nazuha Mansor[1], Mohd Syahril Mohd Zan[2], Intan Faraha A Ghani[1,3] & Sulaiman Abdul Haq Hazman[1]

[1]Department of Science and Biotechnology, Faculty of Engineering and Life Sciences Universiti Selangor, 45600 Bestari Jaya, Selangor, Malaysia
[2]Faculty of Applied Science, Universiti Teknologi MARA, Kuala Pilah Campus, Negeri Sembilan, Malaysia.
[3]Selangor Centre of Research on Sustainable Agriculture (SCORSA), Universiti Selangor, 45600 Bestari Jaya, Selangor, Malaysia

roshani@unisel.edu.my

ABSTRACT

The sustainability and ethical progress of aquaculture is highly dependent on accurate, non-invasive tools to monitor the reproductive maturity of broodstock. The aim of this study was to develop and evaluate a vitellogenin dipstick, a lateral flow immunoassay for the rapid detection of vitellogenin (Vtg), a biomarker of sexual maturity, in female catfish to support Shariah-compliant aquaculture practises. Polyclonal antibodies were generated in rabbits using purified Vtg from *Hemibagrus nemurus* selected for its high Vtg expression. These antibodies were conjugated to gold nanoparticles and applied to nitrocellulose membranes to develop a field applicable dipstick. Plasma samples were collected from four catfish species, *Clarias gariepinus* (mature and immature), *Pangasius pangasius*, *Hemibagrus wyckiodes* and *H. nemurus,* and analysed in triplicate. The Vtg content was quantified by dipstick and competitive ELISA using optimised antibody dilutions of 1:500 to 1:8000 and standard Vtg concentrations of 125–500 ng/mL. SDS-PAGE confirmed the presence of Vtg bands (95–175 kDa) in females and the absence in males. Notably, *H. nemurus* had the highest concentration of Vtg in plasma (84.040 ± 5.33 mg/mL), while *C. gariepinus* (mature) had the lowest (8.539 ± 2.68 mg/mL). The Vtg dipstick showed high sensitivity by detecting Vtg in concentrations of up to 50 ng/mL within 10 minutes and showed a strong correlation with the ELISA and the gonadosomatic index (r = 0.87). The developed test strip is portable, cost-effective (<RM 5 per test) and eliminates the need for synthetic hormones, providing a humane and Shariah-compliant solution for aquaculture. In conclusion, the Vtg dipstick is a promising innovation for non-invasive monitoring of catfish reproduction that supports ethical breeding decisions and contributes to sustainable aquaculture practises.

Key Words: Catfish, Dipstick, Immunoassay, Shariah-Compliant Aquaculture, Vitellogenin

1. INTRODUCTION

Aquaculture contributes significantly to global food security by providing a sustainable source of protein (Franks *et al.,* 2021). However, there is a growing demand for ethical and Sharia-compliant practises in animal protein sources. Conventional methods such as hormone injections, biopsies and laboratory tests are often invasive, expensive and stressful for the fish (Evgenyevich, 2022). Hormone treatments can lead to problems with egg quality, abnormal gonadal development, infertility and ethical concerns, especially when derived from human urine (Hu *et al.,* 2020; Mylonas *et al.,* 2010; Sutriana *et al.,* 2022). This raises questions about halal integrity, as Muslim farmers and consumers are concerned about consuming fish that has been exposed to questionable substances ('mashbooh'). Therefore, non-invasive, Sharia-compliant alternatives are urgently needed to ensure ethical and sustainable aquaculture practises. Vitellogenin (Vtg), a liver-derived precursor of egg yolk protein, is a proven biomarker for sexual maturity (Daud *et al.,* 2016; Kochneva *et al.,* 2023). The aim of this study was to develop and evaluate a portable Vtg-based test stick for the rapid and non-invasive detection of sexual maturity in female catfish.

2. METHODOLOGY

Vitellogenin (Vtg) was purified from catfish plasma by ion exchange and gel filtration chromatography. Polyclonal antibodies were raised in rabbits by injection of purified Vtg mixed with Freund's adjuvant, followed by purification by affinity chromatography and freeze-drying for storage. Colloidal gold nanoparticles were conjugated with anti-Vtg antibodies and applied to a conjugate pad containing sucrose and trehalose. Lateral flow dipsticks consisted of sample pads, conjugate pads, nitrocellulose membranes (with test and control lines) and absorbent pads. Female plasma from *Clarias gariepinus*, *Pangasius pangasius*, *Hemibagrus wyckiodes* and *H. nemurus* was analysed for Vtg by SDS-PAGE and quantified using a competitive ELISA. The Vtg concentrations were correlated with the values of the gonadosomatic index (GSI). Tests were performed in triplicate and data were analysed using a one-way ANOVA and Tukey's post hoc test ($p>0.05$). All procedures followed the Universiti Selangor ethical guidelines (UNISEL/CRIL/EC/0001).

3. RESULTS & DISCUSSION

3.1. Protein characterisation and sex-specific vitellogenin detection

Total plasma protein concentrations varied between catfish species, with *H. nemurus* having the highest (~0.1240 mg/mL) and mature *C. gariepinus* the lowest (~0.0987 mg/mL) (Figure 1(A)). Immature *C. gariepinus* had higher protein concentrations than mature broodstocks, indicating early Vtg synthesis. SDS-PAGE analysis confirmed the presence of a Vtg band (~95-175 kDa) in the plasma of females of all species, while male samples had weak or absent bands (Figure 1(B)). These findings demonstrate that Vtg is a reliable female-specific biomarker for reproductive maturity in catfish.

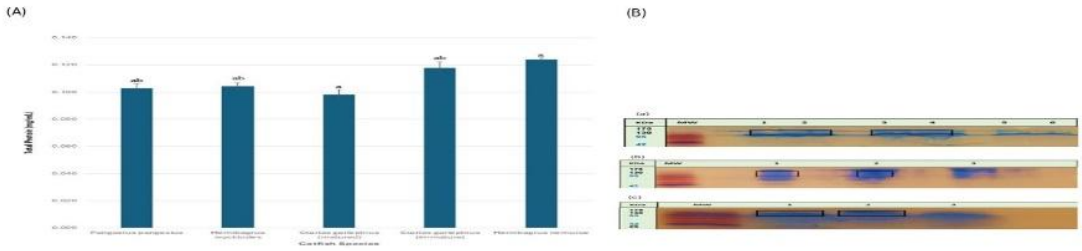

Figure 10: Total plasma protein and vitellogenin expression in female catfish. (A) Mean plasma protein concentration (±SE) in *Clarias gariepinus* (mature and immature), *Pangasius pangasius*, *Hemibagrus wyckiodes*, and *H. nemurus*; (B) SDS-PAGE showing Vtg bands (~95–175 kDa) in female plasma; bands were absent in male samples, confirming sex-specific expression.

3.2. Antibody sensitivity and evaluation of reproductive biomarker

Antibody sensitivity (Figure 2 (A-C)) confirmed strong anti-Vtg binding at low antibody dilutions (1:1000), with higher Vtg concentrations producing stronger absorbance signals, confirming the sensitivity and specificity of the assay. The polyclonal IgG effectively detected Vtg in the range of 125–500 ng/mL. Figure 2 (D) shows a positive correlation between Vtg levels and the gonadosomatic index (GSI), with the highest values observed during the maturation phase. Lower Vtg and GSI values were observed in immature fish. These findings confirm Vtg as a reliable and non-invasive biomarker for reproductive maturity in female catfish.

3.3. Species-specific evaluation of vitellogenin in female catfish

Table 1 shows the species-specific differences in Vtg plasma levels. *Hemibagrus nemurus* had the highest Vtg concentration (84.040 ± 5.330 mg/mL), followed by *P. pangasius* and *H. wyckiodes*. *Clarias gariepinus* (mature and immature) had the lowest values. These differences reflect species-specific hormonal regulation, reproductive stage and liver activity during oocyte maturation (Warren *et al.,* 2021; Villeneuve *et al.,* 2023).

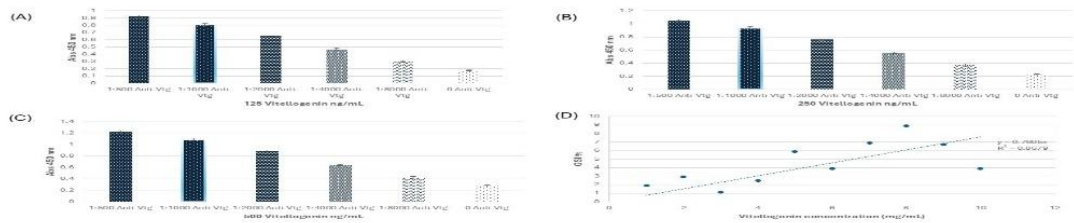

Figure 2: Antibody sensitivity and vitellogenin-GSI correlation in female catfish. (A–C) Antibody sensitivity shows absorption at concentrations of 125, 250 and 500 ng/mL Vtg; strongest binding was observed at dilutions of 1:500–1:1000. (D) The strong linear correlation (R^2 = 0.86) between Vtg concentration and GSI confirms Vtg as a reliable biomarker of maturation.

Table 2 Plasma vitellogenin concentration in female catfish: mean ± standard deviation. Superscript letters indicate statistically non-significant differences at p > 0.05 based on Tukey's post-hoc test.

Catfish species	Vitellogenin concentration (mg/mL)
Clarias gariepinus (mature)	8.539 ± 2.677[a]
Clarias gariepinus (immature)	7.148 ± 2.988[a]
Pangasius pangasius	26.662 ± 3.787[ab]
Hemibagrus wyckiodes	21.958 ± 4.673[ab]
Hemibagrus nemurus	84.040 ± 5.330[b]

3.4. Commercialisation potential and application for Shariah-compliant aquaculture

Table 2 compares Vtg dipstick, ELISA, and conventional methods for assessing the maturity of broodstocks. The Vtg dipstick is inexpensive, fast (~10 minutes), portable and user-friendly, requiring only a drop of blood and no equipment. Unlike ELISA or hormone-based methods, it minimises stress, avoids synthetic chemicals and is in line with halal principles, making it suitable for use in the field (Kampeera *et al.,* 2021; Major *et al.,* 2022). This innovation allows non-experts to carry out on-site inspections, reducing reliance on invasive techniques. Its use supports ethical breeding practises, increases productivity and consumer confidence in Sharia-compliant fish products. The Vtg Dipstick therefore offers a scalable, sustainable solution that combines scientific accuracy with the practical needs of aquaculture.

Table 2 Comparison of vitellogenin dipstick, ELISA and conventional methods

Characteristics	Vitellogenin dipstick	Vitellogenin ELISA	Conventional method
Equipment	Device-free, portable	Requires laboratory equipment	Visual aids or biopsy instruments required
Ease of use	Very simple, one drop of blood, quick result (10 min)	Complex, multi-step, time-consuming	Requires trained personnel, subjective
Stress for the fish	Minimal, non-invasive	Moderate, blood sampling required	High stress; handling of hormones
Accuracy	High; recognises plasma Vtg	High, quantitative	Moderate; based on physical characteristics
Cost	Low (~RM 5/test)	High (~RM 6,000 for set-up)	Variable (labour, hormone costs)
Time to result	~10 minutes	Several hours to a day	Immediate, but low accuracy
Sustainability	Halal, hormone-free, ethical	Less hormone use, but invasive	Spawning induction, Often hormone-dependent

4. CONCLUSION

In this study, a rapid, Sharia-compliant Vtg dipstick was developed for the detection of sexual maturity in female catfish. It showed strong cross-reactivity, high sensitivity (50 ng/mL) and strong ELISA correlation. This tool supports the ethical, non-invasive assessment of broodstock and has the potential for wider field validation and commercial aquaculture applications.

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