

Immersion of nanostructured lipid carriers loaded with 17-alpha methyltestosterone for masculinization of red tilapia (Oreochromis sp.)

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Highlights
Novel masculinization agents for enhancing sex-reversal efficiency in red tilapia by immersion using 17alpha-methyltestosterone-loaded nanostructured lipid carriers (MT-NLC).
MT-NLC showed 140.8 nm avg. size, <0.2 polydispersity index, and -26.9 mV surface charge, with improved stability at cool and ambient temps.
Red tilapia fry exhibited no toxicity to MT-NLC and MT-ethanol (MT-ET) treatments by immersion, with LC50 >800 µg/L and normal gill morphology.
MT-NLC-400 obtained 98.3% males with heightened expressions of dmrt1 and sox9, compared to 54.7% males in control.
Red tilapia farming can use MT-NLC-400 immersion to reduce the dose and duration of MT treatment for masculinization.

Abstract

The present study aimed to develop novel masculinization agents for enhancing sex-reversal in red tilapia (Oreochromis sp.) by immersion administration using 17alpha-methyltestosterone-loaded nanostructured lipid carriers (MT-NLC). Moreover, the surface charge of the MT-NLC was modified with chitosan (CS-MT-NLC) to improve their mucoadhesive property. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to characterize the morphology of the nanoparticles. Furthermore, we assessed the toxicity associated with immersion-induced masculinization in red tilapia. Subsequently, we examined alterations in gill morphology, determined the percentage of masculinization, analyzed their length-weight relationship, and investigated the expression of genes related to sex differentiation. MT-NLC had an average size, polydispersity index, and negatively charged surface of 140.8±2.6 nm, <0.2, and -26.9±0.3 mV, respectively, with higher stability at 4 °C and 25 °C compared to CS-MT-NLC. The release mechanisms of NLC and CS-NLC were regulated by diffusion processes, which were characterized using Avrami's equation. In contrast, the release mechanism of non-encapsulated MT (MT-ET) was governed by burst release kinetic. Furthermore, tests assessing mucin binding confirmed that CS-MT-NLC, with a positive charge, exhibited superior mucoadhesive properties compared to MT-NLC, with a negative charge. Importantly, CS-MT-NLC immersion was found to be toxic to red tilapia fry; therefore, it has been discontinued for further experiments. The immersions of both MT-NLC and MT-ET were non-toxic to red tilapia fry, with an LC50 exceeding 800 µg/L, and the morphological appearance of the gills after treatment was normal. The MT-NLC-400 and MT-ET-800 groups exhibited elevated expression levels of dmrt1 and sox9 compared to cyp19a1a and sox2. These higher expression levels corresponded to significantly higher percentages of males (98.3% and 95.7%) in both the MT-NLC and MT-ET groups compared to the control group (54.7%). The MT-NLC and MT-ET groups displayed a negative allometric growth behavior in red tilapia fry, with a relative condition factor > 1. Adopting MT-NLC-400 immersion treatment for masculinizing red tilapia could serve as a beneficial strategy for commercial tilapia farms to reduce the required dosage and the time needed to treat fish.

Introduction

Tilapia has gained significant importance as a protein source for humans due to its ability to adapt to various environmental conditions, its low feed conversion ratio, and higher growth rate (FAO, 2014). All-male populations are preferred over female/mixed sex populations in tilapia farming due to their numerous advantages, including the elimination of their reproductive potential and issues associated with overpopulation and competition for resources (Kamble et al., 2018; Saha et al., 2022). Steroid hormones, 17alpha-methyltestosterone (MT), have been widely used as masculinization agents for tilapia (Vinarukwong et al., 2018), administered either orally, or via immersion (Basavaraja and Raghavendra, 2017; El-Greisy and El-Gamal, 2012).

Compared to in-feed masculinization techniques, immersion masculinization during the egg and fry stages is the preferred method due to its requirement for lower hormone concentrations (100 µg/L to 10 mg/L) and a shorter treatment period (3 h to 14 days after hatching (dah)) (Gale et al., 1999; Karaket et al., 2023), while maintaining sex-reversal efficiency. Nonetheless, the immersion method still poses certain challenges, notably in attaining a low hatching percentage (Arriegasado et al., 2011), a high mortality rate (Bombardelli et al., 2007) and a lower fry masculinization efficiency (Gale et al., 1999). To tackle these issues, lipid-based nanoparticles were developed and optimized to reduce mortality rates, minimize the required dosage, and shorten treatment duration.

A second-generation lipid delivery system, the nanostructured lipid carrier (NLC), has been utilized for delivering hydrophobic molecules in various applications and administration routes (Chaiin et al., 2022; Dana et al., 2021). Nanoparticle with mucoadhesive properties prolong the contact time with the mucus layer, thereby delaying particle clearance (Charlie-Silva et al., 2018). Chitosan, an effective biopolymer, induces mucoadhesion in lipid nanoparticle (Yostawonkul et al., 2017). Androgen hormones, due to their steroid structure, are insoluble in water (Aziz and Ojumu, 2020). NLCs loaded with steroid derivatives like exemestane and steroidal aromatase inhibitors enhance in vivo oral bioavailability for breast cancer treatment (Singh et al., 2019). Additionally, NLCs loaded steroidogenic drugs such as spironolactone and methylprednisolone aceponate improve the treatment efficiency of steroidogenic molecules via transdermal administration (Kelidari et al., 2016).

Administering exogenous androgen hormones to tilapia fry effectively induces masculinization during the gonadal differentiation period (5–6 dah) (Ijiri et al., 2008). Due to the challenge of morphological sex differentiation under a light microscope during this period, sex-related genes such as dmrt1, sox9, cyp19a1a, and foxl2 serve as markers. Dmrt1 (doublesex and mab-3-related transcription factor 1) significantly increases in the XY gonad after one week, playing a crucial role in testicular differentiation (Ijiri et al., 2008). The sox9 gene (SRY-box containing gene 9), intricately interacting with dmrt1 and SRY, plays pivotal role in testicular differentiation (Li et al., 2014). Cyp19a1a (cytochrome P450 family 19 subfamily A member 1), an aromatase enzyme, is linked to testosterone conversion and ovarian differentiation in female tilapia (Li et al., 2021). The transcription factor Forkhead protein L2 (foxl2) emerges as a crucial regulator in ovarian development, with sex-specific expression steering undifferentiated gonads toward ovarian development in XX gonads during early gonadal differentiation (Ijiri et al., 2008; Kobayashi et al., 2008).

Our prior work demonstrated the efficacy of NLC in delivering 17alpha-methyltestosterone as a sex-reversal agent to tilapia by orally, offering high encapsulation efficiency, thermal stability, and cost-effectiveness while reducing the required hormone dose (Yostawonkul et al., 2023). This study aimed to innovate nanostructured lipid carrier (NLC) particles to enhance sex-reversal efficiency in red tilapia through immersion administration of 17alpha-methyltestosterone (MT). Two NLC types were investigated: MT-loaded NLC (MT-NLC), and surface charge modified with chitosan (CS-MT-NLC). Furthermore, we assessed the potential toxicity and efficacy of immersion-based masculinization in red tilapia, examining gill morphology, sex reversal percentage, analysing the length-weight relationship, and expression of sex-related genes.

Section snippets

Chemicals

The hormone 17alpha-methyltestosterone was purchased from Aldamek Co., Ltd. (Bangkok, Thailand). Sorbitan oleate, ceteareyl alcohol, cocoglucoside, polyoxyethylene [20] sorbitan monolaurate, poloxamer, and glycerol were purchased from Croda (Thailand) Co., Ltd. (Bangkok, Thailand). Ethoxydiglycol was purchased from Myskin Recipe Co., Ltd. (Bangkok, Thailand). Low-molecular-weight chitosan was purchased from Merck Co., Ltd. (Bangkok, Thailand)...

Preparation of methyltestosterone loaded nanostructured lipid carriers (MT-NLC)

As a masculinization agent, 17alpha-methyltestosterone (MT) ...

Physicochemical characterization, morphology, and stability of MT-NLC and CS-MT-NLC

The particle size, PDI, and particle surface charge of MT-NLC and CS-MT-NLC are displayed in Fig. 1A and B. MT-NLC had an average size of 140.8±2.6 nm with a PDI below 0.2 and a negatively charged surface (-26.9±0.3 mV). The optimum surface coating ratio of 1:1 (MT-NLC:CS; v/v) was selected for further investigation since it generated nanoparticles with an average size, surface charge, and PDI of 167.4±4.3 nm, 27.5±0.2 mV, and 0.151±0.02, respectively. Although the surface coating...

Discussions

Lipid nanoparticles have been developed as a hydrophobic molecule delivery system for aquatic animals (Kitiyodom et al., 2019; Suwanbumrung et al., 2023; Swathy et al., 2018) and were successfully synthesized as an androgen hormone delivery system in the present study. The high-energy homogenization could be used to synthesize lipid-based nanoparticles, which improve particle stability and reduce particles size with a narrow size distribution (Kovačević et al., 2020; Silva et al., 2012). In the ...

Conclusions

In the present study, we successfully synthesized nanostructured lipid carriers and chitosan-coated nanostructured lipid carriers loaded with 17alpha-methyltestosterone hormone. MT-NLC had an average size, PDI, and negatively charged surface of 140.8±2.6 nm, <0.2, and -26.9±0.3 mV, respectively, with higher stability at cool and ambient temperatures. The MT-NLC and MT-ET delivered by immersion were not toxic to red tilapia fry with LC50 of >800 µg/L and normal gill morphology. The expression...

CRediT authorship contribution statement

Jakarwan Yostawonkul: Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Sirikorn Kitiyodom: Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Manoj Tukaram Kamble: Writing - review & editing, Writing - original draft, Visualization, Validation, Software, Formal analysis, Data curation. Kittipat Supchukun: Writing - review & editing,...

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jakarwan Yostawonkul reports financial support was provided by Second Century Fund (C2F), Chulalongkorn University, Jakarwan Yostawonkul reports financial support was provided by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), Nopadon Pirarat reports financial support was provided by National Research Council of...

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