



## Potentiality of *Solanum Torvum* as a Feminization Agent on *Anabas Testudineus* Bloch 1792: a Review

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### ABSTRACT

Climbing perch (*Anabas testudineus*) has a high economic value. Females grow faster than males. Sex reversal is one of genetic engineering by manipulating their hormone that is able to change the phenotype from male to female or vice versa. "Cepoka" eggplant (*Solanum torvum*) has been known as a medicinal and kitchen ingredient. Phytochemical test on *S. torvum* showed revealed that this plant contains tannins, saponins, flavonoids, steroids, alkaloids and terpenoids. Solasodine is a steroidal glycoalkaloid compound contained in *S. torvum* which has anti-fertility effects. Besides solasodine, the content of Gosipol in phenol compounds with BM 518.55 Dalton also has been known to be able to inhibit spermatogenesis by reducing the concentration, motility and viability of spermatozoa. The solasodine contained in *S. torvum* has the potential to be used for sex transfer in *A. testudineus*. Based on many studies, the utilize of solasodine from *S. torvum* could decrease the motility of spermatozoa. Even the results of the histological test on spermatogenesis, *S. torvum* gave a positive trend in inhibiting tilapia testicular cells in microscopic observation. Based on it, the chances of successful feminization of *S. torvum* utilizing cepoka eggplant are very high. A factor that must be considered in feminization is the age of the larvae, immersion dosige, immersion time and environment.

**Keywords:** Eggplant, Climbing perch, Sex Reversal, Spermatozoa, Solasodine

### ABSTRAK

Ikan betok (*Anabas testudineus*) memiliki nilai ekonomis yang tinggi. Pengalihan kelamin (*sex reversal*) adalah rekayasa hormon genetik yang mampu mengubah bentuk fenotipe dari jantan menjadi betina atau sebaliknya. Terong cepoka (*solanum torvum*) dikenal sebagai bahan obat dan bahan dapur. Review ini membahas tentang *sex reversal* pada ikan betok, pengaruh kandungan solasodine terong cepoka terhadap fertilitas pada hewan dan efek solasodine pada histologis testis ikan nila. Hasil uji fitokimia terhadap buah terong cepoka menunjukkan bahwa tanaman ini mengandung tanin, saponin, flavonoid, steroid, alkaloid dan terpenoid. Solasodine merupakan senyawa glikoalkaloid steroidal yang terkandung pada terong cepoka dan diduga memiliki efek antifertilitas. Selain solasodine kandungan Gosipol dalam senyawa fenol dengan BM 518,55 Dalton yang diketahui mampu menghambat spermatogenesis dengan menurunkan konsentrasi, motilitas dan viabilitas spermatozoa. Kandungan solasodine yang terkandung pada terong cepoka sangat berpotensi digunakan untuk pengalihan kelamin pada ikan betok. Terbukti dari banyak penelitian yang memanfaatkan kandungan solasodine dari terong cepoka sebagai penurun motilitas pada spermatozoa. Bahkan dari hasil uji histologi pada spermatogenesis memberikan trend positif dalam menghambat sel testis ikan nila secara mikroskopis. Penerapan *sex reversal* pada ikan betok dari penelitian yang terdahulu dengan menggunakan susu dan kedelai juga mudah dan perbedaan rasio kelamin jantan dan betina sangat signifikan, sehingga peluang keberhasilan feminisasi ikan betok dengan terong cepoka sangat tinggi, faktor yang harus diperhatikan yang menunjang keberhasilan feminisasi adalah umur larva, dosis perendaman, lama perendaman dan lingkungan.

**Kata kunci:** Ikan Betok, Sex Reversal, Spermatozoa, Solasodine, Terong Cepoka

## 1. Introduction

Climbing perch (*Anabas testudineus*) or locally known as is betok, is a valuable fish and has important economic value. In the calculation of the utilization rate based on actual production data with the maximum sustainable yield (MSY) value of betok fish resources in the inland waters of Banjar Regency in 2016 was 95.51%. Swamps, marshlands, lakes, canals, ponds, rice fields, pools, shallow pits, and estuaries are all common habitats for this species, thus it can be said that the betok fish species are still exploited below the MSY value, so it is very opportunity to be cultivated. The distribution is wide not only in Indonesia (Akbar et al 2016), but also Malaysia, Vietnam, Laos, Cambodia, Thailand (Piwpong et al 2016), Philippines (Bernal et al 2015), India (Mandal et al 2016) and Bangladesh (Uddin et al 2017).

Climbing perch (*Anabas testudineus*) is a valuable fish and has important economic value. The distribution is wide not only in Indonesia (Akbar et al 2016), but also Malaysia, Vietnam, Laos, Cambodia, Thailand (Piwpong et al 2016), Philippines (Bernal et al 2015), India (Mandal et al 2016) and Bangladesh (Uddin et al 2017). Therefore, commercial cultivation of *A. testudineus* still has a high chance.

One problem of culturing *A. testudineus* was the length of time to reach consumption size. Their slow growth and small size do not support sustainable production in aquaculture systems (Mateen et al 2015). The growth of *A. testudineus* in the same cultivation time is different between female and male. Females *A. testudineus* have a higher growth rate than the male *A. testudineus* (Pal et al 2018; Piwpong et al 2016). To get maximum results, using the sex reversal procedure to enhance the growth of *A. testudineus* is required.

Sex reversal is genetic engineering reversing the genital development from female to male (*masculinization*) or male to female (*feminization*) (Baroiller et al 2016; Dussenne et al 2020). Phenotypic sex reversal may occur simultaneously in the same individual (Falah 2017; Imiuwa, 2020; Li et al 2018). Fish sex orientation was chosen because it was able to provide maximum fish growth. Genital direction is usually carried out with synthetic hormones. The use of synthetic hormones can be harmful to humans and the environment (Emilda 2015). Recently, sex reversal can use natural ingredients such as eggplant (*Solanum torvum*).

*Solanum torvum* has been known as a medicinal and kitchen ingredient (Susilo et al 2016; Wannasiri et al 2017). The results of phytochemical tests on Cepoka eggplant fruit showed that this plant contains tannins, saponins, flavonoids, steroids, alkaloids and terpenoids (Alfarabi et al 2018; Candra et al 2019; Helilusiatiningsih et al 2020; Helilusiatiningsih et al 2019; Karmakar et al 2015; Lajira et al 2019; Rumouw 2018; Susilo & Akbar 2016). This compound can be used as an anti-fertility agent (Hendrawan et al 2019; Wannasiri et al 2017). The amount of Solasodine in the alkaloid content of *S. torvum* is 0.84 % (Fandana et al 2020; Hidayati et al 2014; Susilo & Akbar 2016; Zhang et al 2017). Solasodine is a glycoalkaloid steroidal compound contained in *S. torvum* and is thought to have anti-fertiliz effects in the spermatogenesis process (Mandal et al 2016; Wannasiri et al 2017) which inhibits the development of spermatozoa. In addition to solasodine, the content of Gosipol in phenol compounds with BM 518.55 Dalton has been known to be able to inhibit spermatogenesis by reducing the concentration, motility and viability of spermatozoa (Wulansari et al 2019).

Other previous studies only focused on the study anabas testudineus, sex reversal, and the function of the active compound of *S.torvum* Therefore, this review intends to focus on the use of *S. torvum* for female sex transfer of *A. testudineus* because there is no evaluation related to this. The purpose of this review is to evaluate the potential of *S. torvum* in changing the sex of *A. testudineus* to females (feminism).

## 2. The potential of *A. Testudineus*

The male and female growth of *A. testudineus* which are reared for eight months has different growth rates. The female fish showed absolute weight growth and daily growth rates by 1.48 times and 1.17 times, respectively higher than male. This difference is thought to be due to growth-related sexual dimorphism (Helmizuryani et al 2018). In addition, there are 2 factors that influence growth including internal and external factors. Internal factors are factors that are difficult to control such as heredity, the presence of digestive enzymes, gender, and age (Mandal et al 2016; Riaz and Naeem. 2020), while external factors include food and water quality (Purwati et al 2016). The high survival of *A. testudineus* female is influenced by their migrating from the bottom to the surface area, while the male

prefer to stay under the bottom of the water so that *A. testudineus* female get more food than *A. testudineus* male (Helmizuryani et al 2016).

In an effort to cultivate *A. testudineus*, from a series of previous studies, it was found that *A. testudineus* could be domesticated properly. Domestication is an effort to familiarize wild fish with new environment so that they are accustomed to the human household environment, both in the form of food and their habitat (Helmizuryani & Muslimin 2016; Nurulnadia et al 2020). Fish that have been taken from the wild are acclimatized to suitable conditions for 3 weeks in an aquarium with a capacity of 300 L by considering the fish's condition. They are maintained continuously in natural photoperiod, namely 12 h in light condition and 12 h in dark condition with an efficient oxygen supply through aeration. During the acclimatization process, the water quality must be maintained by renewing water every day (Benjamin et al 2019).

After the acclimatization process, *A. testudineus* are usually cultivated using a tarp pool or concrete pond (Dey et al 2019). By using an intensive fish cultivation method, it is possible to increase fish production by improving water quality that takes into account environmental friendly aspects such as the use of natural materials rather than chemicals in aquaculture water treatment (Handajani et al 2021). Water parameters during cultivation are observed by measuring water temperature, DO, and pH every day using a thermometer, DO meter, litmus paper and  $\text{NH}_3$  test kit, respectively (Midhun et al 2017). During the cultivation process, the feed of *A. testudineus* can be met by providing engineered natural feed such as providing brine shrimp during the rearing process (Dey & Ghosh 2019). *A. testudineus* have died due to several factors, such as smaller fish size and passively moving could not consume any food because almost all the food is eaten by large and active fish (Purwati et al 2016).

In Asia, *A. testudineus* attracts great attention as a precise species for freshwater cultivation. They have a great demand by Asian people and a high market price, 6.00 USD to 7.00 USD when compared to other fish (Mandal et al 2016; K. Singh et al 2019). They have been cultivated in several Asian countries such as India (Midhun et al 2017), China (W. Zhang et al 2019), Malaysia (Nurulnadia et al 2020), Indonesia (Syafutri et al 2018), Bangladesh and Thailand (Piwpong et al 2016). In Indonesia, the price of *A. testudineus* reaches IDR 80,000  $\text{kg}^{-1}$  to IDR 100,000  $\text{kg}^{-1}$  for large fish and IDR

40,000  $\text{kg}^{-1}$  to IDR 54,000  $\text{kg}^{-1}$  for medium size (Sibirian et al 2020). This fish is traded in various forms, such as fresh that can be found in traditional markets, in processed form such as dried fish, fermented fish, and in ready-to-eat forms such as fried fish, grilled fish, and other traditional dishes provided in restaurants (Mustika et al 2020). Therefore, the potential for cultivating *A. testudineus* will be very beneficial for the community besides it can reduce the fishing of *A. testudineus* in nature so that the population can return to stability.

### 3. The potency of *S. Torvum*

One of the advantages of the Indonesian state is the many types of plants found in Indonesia that can be used by the community both as food, spices, medicine and others (Alfarabi & Gupita 2018). Development related to *S. torvum* in Indonesia is very limited because this plant is considered as a wild plant and its use has not been well known by the public so far, this plant is only known as a vegetable, even the public does not really know about the properties of plants as traditional medicines. People generally use *S. torvum* as a mixture of vegetables and herbal medicine (Alfarabi & Gupita 2018; Candra et al 2019; Lajira & Lister 2019; Susilo & Akbar 2016). Due to the information of its benefit, research in both of testing the chemical content to their toxicity related to *S. torvum* has begun to be carried out

Several phytochemical tests revealed that *S. torvum* contains tannins ( $\text{C}_{76}\text{H}_{52}\text{O}_{46}$ ), alkaloids ( $\text{C}_{27}\text{H}_{43}\text{NO}_2$ ) (Helilusiatiningsih et al 2019 ; Alfarabi & Gupita 2018), saponins ( $\text{C}_{27}\text{H}_{42}\text{O}_3$ ) (Helilusiatiningsih & Soenyoto 2020), and flavonoids ( $\text{C}_6\text{-C}_3\text{-C}_6$ ) (Candra et al 2019; Karmakar et al 2015; Lajira & Lister 2019; Rumouw 2018; Susilo & Akbar 2016). From some research results, *S. torvum* has the potential to be used as an anti-bacterial because it has flavonoids, alkaloids and saponins (Lajira & Lister 2019). Alkaloids are estrogenic, whose mechanism of action resembles natural estrogens so that they can bind to estrogen receptors, and the alkaloids contained in *S. torvum* are a type of solasodine (Wulansari et al 2019). The solasodine alkaloid contained in *S. torvum* is about 0.84 % (Fandana et al 2020; Hidayati & Nofianti 2014; Susilo & Akbar 2016). Solasodine, which is a steroid alkaloid, causes changes in the acrosomal membrane of the spermatozoa and decreases the motility of spermatozoa (Wulansari et al 2019).

Many studies have shown that solasodine in *S. torvum* can inhibit the development of spermatogenesis. Solasodine alkaloids are competitive against FSH (*Folicle Stimulating Hormone*) receptors, so that the release of FSH from the pituitary will be disrupted, FSH acts as a mediator to bind androgens in spermatogenesis (Kaspul 2017). Solasodine compounds have activities that can suppress the function of the anterior pituitary to secrete FSH and LH (*Luteinizing Hormone*) through negative feedback on the axis of the Hypothalamus - Pituitary - Testes. The decrease of LH causes a decrease of testosterone production in Leydig cells and the decrease of FSH will inhibit Sertoli cells for synthesizing ABP (*Androgen Binding Protein*). Consequently, spermatogenesis will be inhibited and the quality of sperm will be decreased (Susilo & Akbar 2016).

Based on various research about the effects of *S. torvum* on sperm, there are different results depending on the objectives of the study, from observing sperm movement until the protein content in sperm. The absence of FSH due to solasodine contained in *S. torvum* causes inhibition of spermatogenesis activity that retard the division of spermatogenic cells. Based on it, a decrease in the number of spermatogenic cells effect on lower the spermatogenesis index (Kaspul 2017). The decrease of spermatozoa cells could be identified by the empty spaces in the tubules because the spermatogenesis process did not occur completely. Microscopic study revealed that tissue damage is seen during the spermatogenesis process caused by *S. torvum* extract (Fandana et al 2020).

#### 4. Sex Reversal

The advantage of sex reversal is to increase the production of fish because the cultivation system is mono sex (Kautsari et al 2017). Cultivation of fish with single male or female sex systems provides higher production yields than mixed cultivation (Ghosal et al 2015). The use of sex reversal has been carried out in fish and shellfish including tilapia (*Oreochromis niloticus*), giant prawns (*M. rosenbergii*), gundara fish (*Anoplopoma fimbria*), tiger puffer (*Takifugu rubripes*), grouper (*Epinephelus coioides*), goldfish (*Cyprinus carpio*), and Banggai Cardinalfish (*Pterapogon kauderni*) (Ren et al 2018; Sun et al 2017; F. Wang et al 2017; X. Zhang et al 2017; Safir et al 2020). In addition, in the cultivation of ornamental fish, mono sex is also

useful in the beauty and uniqueness of the fish's fin shape. For example, male guppies are more attractive in color than female guppies (Malik et al 2019; Nurlina et al 2016), male Rainbow fish are more attractive in color than females (Setiawan et al 2019), male betta fish are more in demand because they have special bright and beautiful colors (Hidayani et al. 2016; Siregar et al 2018).

One of the synthetic ingredients that is usually used in sex transfer is Estradiol-17 $\beta$  which has strong estrogen and has been applied to the feminization of *Takifugu rubripes* (Ren et al 2018). Another synthetic material that has been used in sex transfer is Letrozole (LET). It contains triazoles which can inhibit the activity of cytochrome P450 aromatase, so that it can affect the level of estrogen production (F. Wang et al 2017). The 17- $\alpha$  methyltestosterone is a synthetic matter used to male genitalia conversion in some fishes including *Xiphophorus maculatus* (Khiabani et al 2016), *Gobiocypris rarus* (Gao et al 2015), *Clarias gariepinus* (Robert et al 2019) and *Oreochromis niloticus* (E. Singh et al 2018).

Natural feed that can replace synthetic materials in sex reversal have been studied by many researchers, one of which is goldfish testes for sex reversal of *O. niloticus* to be males (Ranjan et al 2015). Other natural ingredients include *Basella alba*, *Tribulus terrestris*, *Mucuna pruriens* and *Asparagus racemosus* root for male sex conversion (Ghosal et al 2015; Pal et al 2018). A study by Turan (2017), the use of *Glycyrrhiza glabra* root could switch the sex of *Poecilia reticulata*. Other natural ingredients from animals, bull testicles are used for masculinization of tilapia (Yustiati et al 2018). In addition, bull testes and cow testes can also replace the use of synthetic hormones in the masculinization of betta fish (Hidayani et al. 2016).

Sex reversal has two methods consisted of direct therapy (hormone) and indirect therapy (chromosome engineering). The method of hormone therapy has three methods including by the feed (oral), immersion (dipping) and injection. Oral method is conducted by mixing hormones in the feed and practically, this method is very easy to be applied (Ayuningtyas et al 2015). For example, estradiol-17 $\beta$  hormone directly can be used by oral administration (Firmansyah et al 2017). The oral method is not only simple to be applied but it also has a disadvantage such as the treatment feed could not be eaten directly by fish so that the hormone given are easily lost. Besides, the hormone that enter through

digestion can be degraded by digestive enzymes. Dipping method is conducted by giving hormones in the water cultivation for a certain time. The immersion method consists of three types including the immersion of the embryo, larvae and the immersion of the broodstock. The dipping method has been examined by a lot of study, but in practice it requires a sufficient amount of hormone to immerse the embryo, larva or broodstock. Hormones can be given by soaking embryos, larvae or broodfish with a certain dose (Dwinanti et al 2018; Grandi et al 2007). The injection method is carried out by injecting hormones into brood fish that have been in mature gonads condition to produce larvae in mono sex. This method is very efficient and inexpensive because it only requires a few milliliters of hormones and the hormones enter the broodstock body directly. However, this method is still considered inadequate because it has to use special tools and materials as well as expertise in injecting techniques. A study by Zahri et al (2017), hormone injection in the brood of eel was carried out intramuscularly (IM) at the dorsal fin with  $1 \text{ mL kg}^{-1}$  of estradiol and methyl testosterone combined with hCG and anti dopamin.

### Sex reversal mechanism

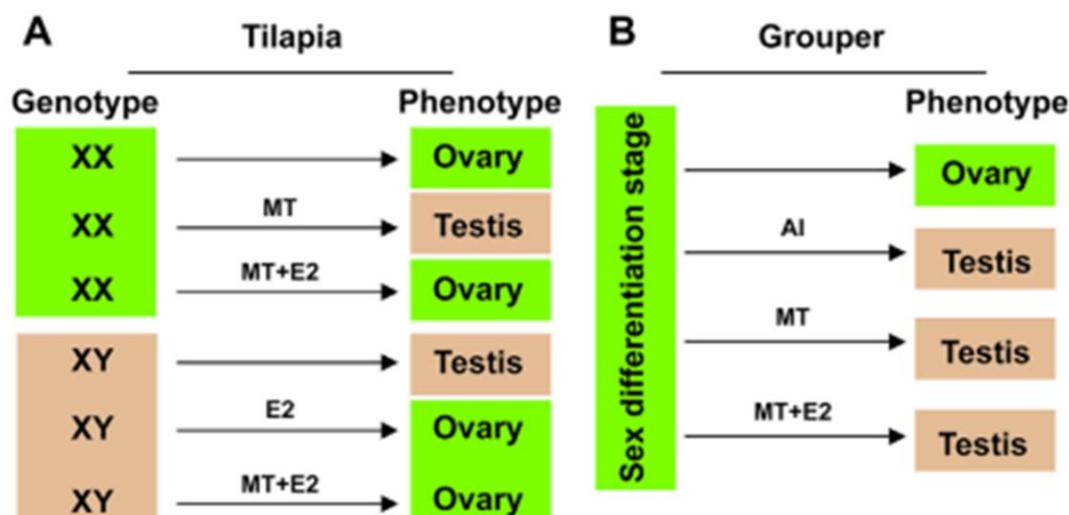
The transfer of sex from female to male or male to female can be exemplified by the hormones  $17\alpha$ -methyltestosterone (MT) and estradiol- $17\beta$  in the following treatment Figure 1.

(A) In gonochoristic tilapia, treatment of

$17\alpha$ -methyltestosterone MT induces testicular development in XX tilapia. Meanwhile, the estradiol- $17\beta$  (E2) induces ovarian development before gonadal differentiation in XY tilapia. Simultaneous treatment of XX and XY fish with MT and E2, respectively, before sexual differentiation process resulted in all females fish. (B) In hermaphrodite grouper spot orange, treatment with aromatase inhibitor (AI) or MT induces testicular development. In contrast to tilapia, the treatment of MT and E2 groupers stimulated the masculinization of fish gonads (Chen et al 2016; Li et al 2019; Q. Wang et al 2018).

### 5. Feminization in *A. testudineus*

During the process of sex differentiation, sex direction in fish involves hormonal processes. It can be applied because sex determination in fish is a flexible process (Piferrer and Guiguen 2008), during the differentiation period. However, it is very difficult, even impossible after the differentiation period has passed (Nakamura et al 2003). Therefore, the study of sex differentiation in *A. testudineus* should consider the timing of fish sex differentiation. That information will very useful as a basis for determining the age of the fish and the the best way to apply the MT hormone in the sex reversal process (oral or immersion). Sex differentiation is a process of transformation from undifferentiated gonads to testes or ovaries, which are influenced by some factors such as genetic, environment, or both so that phenotypically determine male or female



**Figure 1.** Effect of estrogen and androgen administration on fish gonad differentiation.  
Source: M. Li et al (2018)

(Penman and Piferrer 2008). The dosage and timing of MT hormone administration in fish determine the success of the sex reversal process. Feminization using the estradiol-17 $\beta$  hormone orally or immersion in *A. testudineus* larvae of 14 d post-hatch (hpt) for four weeks resulted in 92.6 % to 100 % of female. The survival of treated *A. testudineus* by oral sex reversal (43.33%) was higher than through immersion (36.67%) (Piwpong et al 2016).

The sex reversal of *A. testudineus* larvae could also use natural matter such as cow's milk by soaked for 10 h which has a very significant effect on *A. testudineus* larvae because of the estrogen content in cow milk. According to Stelkens & Wedekind (2010), fish that are exposed to estrogen can change their gonads to become female, and even cause infertility. Therefore, it is necessary to have the correct dosage for its application. Estrogen can also stimulate in accelerating vitellogenesis in fish female (Pinto et al 2014). The cow's milk contains 0.093 mg mL<sup>-1</sup> of estrogen, while synthetic hormone contains 0.065 mg mL<sup>-1</sup> of estrogen (Grgurevic et al 2008). In addition, generally, a high dose of immersion requires a short soaking time and vice versa.

The gonad of *A. testudineus* female is in the form of a transparent gel like soft tissue containing egg granules. The results of staining using acetocarmine showed that the egg cell is rounded and there is a faded cell nucleus surrounded by red cytoplasm. Whereas in the male gonads, there are no egg granules, but there are delicate lines that spread with spermatozoa cells in the form of fine dots (Hidayat 2016). The initial process of sex formation occurs when the larvae are 6 d to 14 d old. During the process of forming fish sex will be influenced by several factors, namely age, size, length of treatment, fish species, genetics, type of hormone, hormone dosage, and treatment time (Helmizuryani et al 2016).

Administration of estrogen at this early stage relates to the early stage of larval development when the gonads are in a labile period for external hormonal influences. The end of this unstable period has been not known certainly, so it is suspected that this period always occurs in the larval stage. There are several types of estrogen including animal estrogen, plant estrogenic, and synthetic estrogenic (Pinto et al 2014). The animal estrogen has good estrogen bonds so that estrogen play a role in the physiological formation of fish reproduction and is able to improve immune function. In the early stages of gonad development of larvae, sex has not been

yet fully formed. In this period, the gonads can be directed to male or female by external hormones. Moreover, estrogen can help in the formation of the female sex. There are two factors that influence the differentiation or formation of the sex ratio, namely internal genetic and environmental (Ariyanto et al 2010). On the other opinions, age of organisms, immersion time, dosage, and temperature are all factors that can also influence fish sex differentiation (Ariyanto et al 2010; Shao et al 2017).

Bioactive compounds contained in *S. torvum* fruit come from the steroid, alkaloid, isoflavonoid, triterpenoid, xanthone, tannin and flavonoid class. Flavonoids are the most diverse and widespread secondary metabolites found in plants (Kata, 2008). For plants, flavonoid compounds play a role in self-defense against pests, diseases, herbivores, competition, interactions with microbes, seed dormancy, protection against UV radiation, signal molecules in various transduction pathways, as well as signal molecules in male pollination and fertility (Kata, 2008). Flavonoids have many types of derivative compounds, from complex to simple. One of the flavonoids that has a potential to affect fertility is solasodine. Solasodine has activities that can suppress the function of the anterior pituitary to secrete FSH and LH (Susilo & Akbar, 2016; Kata, 2008). It has been proven that *S. torvum* extract could decrease the number of spermatozoa and sperm motility for 10 d (Rafiq et al 2013).

Solasodine, an estrogenic compound derived from *S. torvum*, can inhibit the balance of hormone gonadotropins in the hypothalamus and the secretion of LH and FSH in the anterior pituitary which cause the decrease of sperm cell production (Rafiq et al 2013). In male, LH plays a role in reproductive function by modulating Leydig cell and steroidogenesis (Rahman et al 2013). Testosterone is secreted by Leydig cells which has functions for the initiation of male sex differentiation, pubertal androgenization, and fertility. In the testes, LH receptors are expressed on Leydig cells during fetal development and after birth to adult (Rahman et al 2013). Although, mostly LH receptors are expressed on the gonads, but they are also found in the prostate, epididymis and seminal tract (Sridevi, 2011).

The administration of *S. torvum* fruit extract containing flavonoid compounds has an effect on the decrease of the spermatid formation. This decrease is likely due to disruption of the meiotic division during spermatogenesis. The primary spermatocytes

perform the first meiotic division to form secondary spermatocytes and when they perform the second meiosis to become spermatids (Sridevi, 2011; Rahman et al 2013). The decrease in the number of sperm is also suspected to be related to the sperm transport mechanism in the male reproductive tract.

The testosterone hormone is an important sex hormone in male. This hormone is secreted by Leydig cells under the influence of LH. Leydig cells contain high levels of enzymes needed for the direct conversion of cholesterol to testosterone (Sridevi, 2011). Part of the testosterone production will be secreted into the blood and circulated to target cells. Some of them will enter into seminiferous tubules and play an important role in the spermatogenesis process. In male blood plasma, testosterone is the main androgen which is generally found bound to a protein molecule (Binding Protein) such as about 40% bound to albumin protein, about 60% bound to globulin protein. In addition, the bind of testosterone and globulin protein become sex hormone binding globulin (SHBG). These bonds make it easier for androgens to enter the target cells and provide physiological effects.

The administration of solasodine isolated from *S. torvum* fruit played a role in male rat infertility which was indicated by the decrease of number and motility of spermatozoa. According to Rafiq et al (2013) extract of tamarillo (*S. bataveum*) which contains flavonoids caused morphological damage and reduced the motility of mice spermatozoa. The application of *S. nigrum* L, which contains the same content as *S. torvum* fruit, had an effect on reducing the motility of mice spermatozoa (Susilo & Akbar, 2016). *S. torvum* contains solasodine flavonoid compounds that could be used as anti androgenic agent (Rafiq et al 2013). Solasodine compounds can interfere the activity of the ATP-ase enzyme in the sperm cell membrane. ATP-ase is present in the middle of the sperm tail which is to maintain internal homeostasis for sodium and potassium ions (Badkoobeh et al 2013). According to Elia et al (2015), sperm motility is depend on the composition of sodium and potassium ions. Based on it, if the activity of the ATP-ase enzyme is disrupted, the homeostasis of sodium and potassium ions will be disturbed as well so that sperm motility will also be disturbed.

## 6. Conclusion

The solasodine contained in *S. torvum* has the potential to used for sex reversal in *A.*

*testudineus*. Many studies have proved that the utilize of solasodine from *S. torvum* could decrease the motility of spermatozoa. In fact, the results of histological tests on spermatogenesis gave a positive trend in inhibiting tilapia testicular cells under microscopic observation. The application of sex reversal to *A. testudineus* from previous studies using milk and soy was also easy and the difference in male and female sex ratios were very significant. Based on that, the chances of successful feminization of *A. testudineus* using *S. torvum* are very high. Many factor that must be considered to support the success of feminization are the age of the larvae, immersion dose, immersion time and environment.

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## Conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this article

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