Effects of long term feeding of Quillaja saponins on sex ratio, muscle and serum cholesterol and LH levels in Nile tilapia (Oreochromis niloticus (L.))

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Abstract

Seventeen-day-old Nile tilapia fry were fed a standard diet (C) or diets containing 50–700 mg kg⁻¹ Quillaja saponin (QS) extract (groups S50, S150, S300, S500 and S700). After the first 8 weeks, 30 randomly selected tilapia from each of the treatments were placed in separate aquaria and fed the standard diet without saponins from then on (these were designated S50/C, S150/C, S300/C, S500/C and S700/C). The fish grew from an initial average weight of approximately 30 mg to a final average weight of 79 g during the 6-month feeding period. The difference between the average weight of C-fed tilapia and the treatment with the highest average weight after 6 months was 53.5%. The sex ratio of tilapia in the saponin-fed groups deviated from the normal 50:50 male:female ratio, with the S700 group showing a significantly higher number of males. Quillaja saponin stimulated LH release from dispersed tilapia pituitary cells in vitro. This effect was abolished in the presence of dilute calf serum. Serum LH values did not show any diet-dependent trend in either male or female tilapia in vivo. In both continuously saponin-fed and only-initially saponin-fed groups, the average serum (but not muscle) cholesterol levels in males showed an increasing trend (R² values of 0.62 and 0.69) with increasing dietary saponin level. It was concluded that dietary QS has the potential to change the sex-ratio in favour of males. More investigations are required to determine the mechanism of action and the optimum dietary level of QS for maximum effects.

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Keywords: Nile tilapia; Quillaja saponins; Sex-ratio; Growth; Leutinizing hormone; Cholesterol

1. Introduction

The saponins are naturally occurring surface-active glycosides mainly produced by plants, but also by lower marine animals and some bacteria (Yoshiki et al. 1998; Riguera, 1997). Their ability to form stable, soap-like foams in aqueous solutions attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin), which may be triterpenoid or steroid in nature. One example of an extensively studied group of triterpenoid saponins is produced from Quillaja saponaria, a tree native to the Andes region. Water
extracts of the bark of this tree has been traditionally used by shamans as an overall curing agent. When present in the diet of animals, saponins are believed to have several negative effects. For example, dietary saponins derived from different plants have been held responsible for depression of feed intake, reduction in weight gain, accentuation of ruminant bloat, photosensitization (Cheeke, 1996), inhibition of the active uptake of nutrients (Johnson et al., 1986) including vitamins (Jenkins and Atwal, 1994) and minerals (Southon et al., 1988) in the intestine, lowering of protein digestibility (Shimoyamada et al., 1998), and for inducing infertility (Tewary et al., 1973; Quin and Xu, 1998). On the other hand, saponins have also long been known to possess properties useful to man as they are the active components in a large number of traditional ‘herbal medicine’ preparations. There have been reports of antiviral activity of saponins from Glycyrrhiza radix, cholesterol lowering activity of saponins from soybean, immunostimulant activity of saponins from Quillaja saponaria Molina, and hypoglycaemic and anti-diabetic activity of saponins from fenugreek (Kensil, 1996; Petit et al., 1993). Partially purified Quillaja saponaria Molina saponin preparations such as Quil A, have found wide-spread use in veterinary vaccines (Kensil, 1996). Saponins are important in human nutrition as well because of their widespread occurrence in food constituents such as legumes.

Tilapia aquaculture is and will continue to be important, particularly for the lesser-developed countries in the tropics (FAO, 2001). A major drawback of tilapia as a culture fish is their prolific reproduction, resulting in a harvested fish size, which is unacceptable in many markets. Treatment with steroid hormones has been applied to produce all-male populations, in order to circumvent this problem. Because of environmental and consumer health concerns (over 99% of hormones administered through the diet are released into the water in less than 24 h; see review by Pandian and Sheela, 1995), this treatment is prohibited in many countries.

Tilapia are also otherwise valuable as an important experimental vertebrate model (Anken et al., 1993) and as a potential source of pancreatic islets for possible transplantation into diabetics (MacKenzie, 1996). Studies on the physiology of these fish are, therefore, of high scientific interest.

In previous experiments, we observed that dietary Quillaja saponins (QS) stimulate growth in carp (Francis et al., 2002) and in addition inhibit egg production by female Nile tilapia (Francis et al., 2001 and unpublished). The objectives of the current experiment were to study the effects of QS in the diet on sex ratio in Nile tilapia fry, and when tested on LH secretion from tilapia pituitary cells in vitro. Observations were also made on the level of muscle and serum cholesterol levels of the fish.

2. Materials and methods

2.1. Experimental feeds

There were six experimental diets designated C (control), S50, S150, S300, S500 and S700 prepared from the same basal diet to ensure uniform composition. The basal diet contained approximately 40% protein, 10% lipid, 10% ash and had 20 kJ g⁻¹ gross energy on a dry matter basis. The ingredients were as previously reported (Francis et al., 2001). The basal diet was pelleted (to approx. 2 mm diameter) with no additions in the case of the control feed (C). Saponin (QS; Sigma no. S-2149, Sigma, St. Louis, MO, USA), dissolved in demineralized water, was mixed thoroughly (at 50, 150, 300, 500 and 700 mg kg⁻¹ basal diet in the S50, S150, S300, S500 and S700 diets, respectively) with the powdered feed using a mixer before pelleting. The moist pellets were frozen, freeze-dried and stored in a freezer (−18 °C) until use. For the in vitro LH production experiments from dispersed tilapia cells, QS, and its purified form (QS-P, Sigma no. S-4521) was used.

2.2. Experimental fish and feeding regime

Approximately 600 14-day-old tilapia fry were obtained from the ‘Institut fur Tierzucht und Hausstiergenetik’, Georg-August Universitat, Gottingen, Germany. They were fed a commercial (‘Tetra mini’) flaked feed for 3 days to allow them to recover and acclimatise to conditions in the laboratory. They were then divided into seven treatment groups of 70 fish each. Two of the seven groups were fed control (C) feed and each of the five remaining tanks were provided the S50, S150, S300, S500 and S700 diets, respectively. The fish were fed at the rate of 20% of body mass till they reached 0.8 g, then 12% of body mass till they
weighed on average 3.5 g each, followed by 6% of body mass till the average weight was approximately 12 g. Afterwards, they were fed at a level of 20 g per kg metabolic body mass (kg^0.75) and was further reduced, when they reached approximately 25 g body mass, to 10 g per kg^0.8. After 8 weeks of feeding the experimental diets, 30 tilapia from each of the five treatment groups were placed in separate tanks and fed the C diet. These groups were designated S50/C, S150/C, S300/C, S500/C and S700/C, respectively, based on the diet of their original group. Simultaneously, the two control groups were split each into two separate groups to equalize tank-density.

During the experiment, the fish in each treatment were kept in 75-l aquaria that were part of a main recirculating system, under a 12 h-light/12-h darkness regime. The water in the aquaria with saponin-containing feed was allowed to flow through to avoid possible contamination in the recirculating system. The fish were weighed once every two weeks and the feed ration adjusted according to body mass. Water flow was adjusted to keep oxygen saturation constantly above the acceptable levels (pH: 7–7.9; total NH3: 0.1–0.2 mg l⁻¹; nitrite: 0.07–0.1 mg l⁻¹ and nitrate: 1–3 mg l⁻¹) throughout the experiment.

### 2.3. Measurements and analyses

Three male and three female fish from each group were taken out after 5 months to draw blood for hormone analysis. Blood was drawn from the haemal arch of each fish using disposable syringes and was stored on ice until the serum was separated by centrifugation (10 000×g for 5 min). The serum was stored at −20 °C until LH radioimmunoassay (RIA). These fish were then discarded.

After approximately 6 months (June 2000 to November 2000) of feeding the experimental diets, the fish in each treatment group were first collectively weighed and the number of males and females in each group counted. Sexing was based on the shape of the genital papilla, which is broad in females and pointed in males. The male fish also produced milt upon gentle squeezing of the abdomen. The Specific Growth Rate (SGR) was calculated using the formula \( \left( \frac{\ln(W_f) - \ln(W_i)}{t} \right) \times 100 \), where LN(W₀) and LN(W_t) are natural logarithms of initial and final weights and \( t \) is the time interval in days.

Five males and five females were again randomly selected from each group. The sample number was kept low in order to have enough fish for subsequent breeding experiments. Blood was drawn from the sampled fish as previously described. The serum was stored at −20 °C until RIA and cholesterol assay. Serum cholesterol was measured using a cholesterol estimation kit (Roche Diagnostic, Mannheim, Germany). Five grams of muscle tissue were then taken from each fish and stored at −18 °C for determining muscle cholesterol content (using a cholesterol estimation kit; Boehringer Mannheim, Germany).

### 2.4. In-vitro stimulation of LH secretion

Pituitary cells were dispersed according to Levavi-Sivan and Yaron (1992) and Levavi-Sivan et al. (1995). Briefly, pituitary cells from 80 to 100 fish were collectively dispersed, counted and then plated at 100 000 cells per well of 96-well plate, in 0.2-ml medium (M199, 10% fetal calf serum (FCS), 10 mM HEPES, 1% antibiotics [Pen-streptomycin suspension]; Biological Industries, Beit Ha’emeq, Israel), and cultured for 4 days at 28 °C under 5% CO₂. On the fourth day, the cells were rinsed twice with medium before addition of the test substances in stimulation medium (M199, 1% antibiotics, 0.1% BSA), for 5 h. Salmon GnRH (Trp, Leu²-LHRH; sGnRH) and QS were dissolved directly in the medium and were prepared fresh. The concentration of sGnRH used was 100 nM, the most effective dose in raising taGtH levels in dispersed tilapia pituitary cells (Levavi-Sivan et al., 1995). 20% FCS was added to the stimulation medium instead of the BSA to test the activity of QS in the presence of serum.

### 2.5. Radioimmunoassay

The gonadotropin of tilapia (taGtH), possibly representing the tilapia LH was determined in the medium by specific RIA according to Levavi-Sivan and Yaron (1992). The second antibody employed was donkey anti-rabbit IgG bound to magnetizable compound (Amerlex-M, Amersham, UK). The sensitivity of the assay was 0.5 ng/tube; the intra- and interassay coefficients of variation were 7.3% and 14%, respectively. The taGtH was measured by homologous RIA, according to
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>SGR (% per day)</th>
</tr>
</thead>
<tbody>
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<td>64.3</td>
<td>3.00</td>
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<tr>
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<tr>
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<td>23</td>
<td>65.2</td>
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<td>3.00</td>
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</tr>
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<td>S700</td>
<td>26</td>
<td>94.3</td>
<td>3.21</td>
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</table>

C-tilapia fed control feed; S50/C to S700/C-tilapia fed diets containing 50, 150...up to 700 ppm Quillaja saponins for 8 weeks (week 3 to week 11) followed by control; S50 to S700-tilapia fed diets with 50, 150...up to 700 ppm Quillaja saponins throughout.

Bogomolnaya et al. (1989), using taGtH purified from pituitaries of adult fish during the spawning season. It is presumed, therefore, that this RIA measures the maturational GtH II (=LH) as defined for salmon (Suzuki et al., 1988a,b). Hormone levels were expressed as the ratio between the basal secretion and the secretion after stimulation.

2.6. Breeding experiment

From the remaining fish, one male and three female fish from each treatment at a time were kept in breeding aquaria and observed for 3 months. During this period, they were maintained on the same diets as previously. Female fish killed by the males were immediately removed and replaced as far as possible. All other conditions, such as water-quality parameters, light–darkness regime, etc., were also kept identical.

2.7. Statistical treatment

The deviation of the proportion of males and females from the expected ratio was tested by chi-square test. All other data were subjected to ANOVA and statistical comparisons between treatments were made using Duncan’s multiple range test (Statistica for Windows, release 5.1 H, 1997 edition). The significance of observed differences was tested at \( P < 0.05 \).

3. Results

3.1. Feeding and growth

Fish in all groups consumed all of the feed provided and did not show any abnormal behavior during the experimental period. In the S700/C tank, accidental blockage of the water inlet tube resulted in the death of some fish. In other tanks, there were scattered deaths of one or two fish when they either jumped out of the aquaria or were killed by other fish. Except for the accidental deaths in the S700/C tank mortality was isolated and spread over the different treatments and no linkage could be observed with any of the treatments. Dead fish were removed as soon as they were noticed.

The average final body weight of fish in each treatment group is shown in Table 1. The average body weights of the treatment groups tended to be higher than controls. Among the treatment groups, the fish that received the saponin-containing diet throughout grew better than those that were put on the control diet after 8 weeks. In both of these groups the fish which had once received or continued to receive the S150 diet, had the highest specific growth rate (Table 1). Whether the difference in growth between different treatments was statistically significant could not be ascertained since all the fish in one aquarium were weighed together.

3.2. Sex ratio

The proportion of males and females in the treatment groups is given in Fig. 1. There were irregular sex ratios in the treatment groups compared to controls (Table 2). The number of males was higher in some of the groups that received the saponin-containing diets. Only the S700 group deviated significantly from the expected 50:50 ratio (Table 2). The control group had close to the typical gender ratio.

3.3. Serum LH level

The measurements of LH level in the serum of 5- and 6-month-old male and female tilapia did not yield uniform results. The deviation in values
Fig. 1. Proportion of males and females (phenotypic) among the treatment groups at the end of 6 months.

in the individual groups (3 or 5 fish) were very high, but there were still some differences among treatment groups (Table 3). However, no dietary saponin-dependent trend or pattern of change in LH could be noticed. The hormone levels were higher in males than in females at the age of 5 months. The serum level in males increased two- to three fold from the level at 5 months to that at 6 months. In the 6-month old female fish, the LH increased several-fold during this interval, reaching levels approximately double those of the 6-month-old males.

3.4. In vitro stimulation of LH secretion by QS

The effect of QS on taGtH release was examined using tilapia pituitary cells in primary culture. Exposing the cells for 5 h to QS resulted in a moderate increase in LH release similar to that evoked by sGnRHa. However, the more purified QS (QS-P; 10, 100 or 200 μg/ml) evoked a dose-dependent increase in LH output which was significantly higher than that evoked by 10 nM sGnRHa (Fig. 2a). To determine whether QS can alter the cell response to GnRH, both substances were added concomitantly. The selected saponin concentration was 10 μg/ml to allow for an additive effect. When QS and sGnRHa were added simultaneously, LH release was not significantly different from basal, however, when QS-P and sGnRHa were added simultaneously, gonadotropin release was significantly higher than the basal- and GnRH-induced release (Fig. 2b). Exposure of the cells to 10 μg/ml saponin, in the presence of 20% FCS, abolished its stimulatory effect (Fig. 2c).

3.5. Serum and muscle cholesterol

There were no significant differences between serum cholesterol levels in the treatment groups. The cholesterol content in the serum of female fish was approximately 50% higher than that of males. In both continuously saponin-fed and only-initially saponin-fed groups, the average serum cholesterol levels in males showed a steadily increasing trend, from C to S700/C or C to S700 (Fig. 3). The coefficient of determination values of regression lines fitted to the average values were 0.62 and 0.69, respectively. No such trend
Table 2
χ² and P values of the male–female distribution of tilapia in the various experimental treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of males</th>
<th>Number of females</th>
<th>Total number</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>17</td>
<td>32</td>
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<td>0.72</td>
</tr>
<tr>
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<td>31</td>
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<td>0.59</td>
</tr>
<tr>
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<td>14</td>
<td>16</td>
<td>30</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td>C</td>
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<td>15</td>
<td>30</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
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</tr>
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Each of the calculated chi-square values were compared against the respective P-values to determine significant deviations from the expected 1:1 ratio. The P-value is less than 0.05 only in the case of the S700 group. C-tilapia fed control feed; S50 to S700-tilapia fed diets containing 50, 150,... up to 700 ppm Quillaja saponins for 8 weeks (week 3 to week 11) followed by control; S50 to S700-tilapia fed diets with 50, 150... up to 700 ppm Quillaja saponins throughout.

3.6. Breeding trials

The dominant males in the S150/C, S500/C and S700/C groups were extremely aggressive and killed all the supplied females within a very short time in the breeding tanks, and hence no observations could be made on breeding in these groups. One female each in the S300 and S500 groups spawned once immediately after being placed for breeding, but the eggs did not hatch and were spit out by the females after 2 days; there was no further spawning for the following 3 months. Three female tilapia in the control group and one in the S300/C group spawned after ~4 weeks and produced fry. The mouth-brooding fish were removed from the breeding aquaria and were replaced as far as possible.

4. Discussion

Dietary QS at a level of 3000 mg kg⁻¹ have previously been reported to depress feed intake and growth in Chinook salmon and rainbow trout (Bureau et al., 1998). Significant intestinal damage was caused in both species at a dietary QS level
Fig. 2. The in vitro effect of *Quillaja saponin* (QS) or purified QS (QS-P) on LH release from dispersed tilapia pituitary cells (LH denoted as ratio between basal secretion and secretion after stimulation). (a) Cells were treated for 5 h with either 100 nM salmon GnRH (GnRH) or with various concentrations of the saponin (10, 100 or 200 μg/ml). (b) Release of LH by cultured tilapia pituitary cells stimulated by 100 nM salmon GnRH (GnRH), 10 μg/ml QS, or a mixture of both compounds. (c) Dispersed tilapia pituitary cells were cultured in the absence (basal) or presence of 20% fetal calf serum (ser), or with 10 μg/ml QS (sap) or QS in the presence of serum (sap+ser). Each value is the mean ± S.E.M. of three different wells from three or four independent experiments.

of 1500 ppm. In the current experiment, where the body weight of tilapia increased from 30 mg to ~100 g, QS did not cause suppression of feeding or any other apparent abnormal behaviour up to a dietary level of 700 mg kg⁻¹. The initial feeding rate of 20% of body weight was higher than the 12–15% recommended by Shelton et al. (1981). The fish in the saponin-fed groups had higher absolute specific growth rate (SGR) than controls. This was in agreement with our previous observation of a growth promoting effect of dietary QS in tilapia (Francis et al., 2001). Oral application of other androgenic substances has also been previously found to have a positive anabolic effect in tilapia (Tayamen and Shelton, 1978; Macintosh et al., 1988 also see review by Pandian and Sheela, 1995), particularly at steroid doses that were sub-optimal for sex inversion. However, growth depression occurred invariably over the long term (see review by Pandian and Sheela, 1995). The average SGR values of tilapia that received a saponin-supplemented diet throughout were higher than those receiving saponins only for the first 8 weeks. In both groups, maximum growth was achieved at the 150 mg kg⁻¹ level of supplementation. As the fish in each aquaria were collectively weighed there was no statistical treatment of the growth data. In our previous experiment (Francis et al., 2001) tilapia fed 150 mg kg⁻¹ saponins had significantly higher growth rates 3 weeks after feeding started. Tilapia fed 300 mg kg⁻¹ QS showed a significantly higher body weight gain after 14 weeks of feeding in that experiment. Results of the current experiment seemed to corroborate the growth-promoting effects of dietary QS.

The sex ratio of tilapia in the saponin-fed groups deviated from the normal 50:50 ratio, with the S700 group showing a significant deviation from this pattern in favour of males. Deviation from the normal sex ratio was also evident in the treatment groups that were fed saponins only for the first 8 weeks, suggesting that sex-inversion occurs during this initial period. The first 21 days have been judged the most effective period for sex reversal in tilapia fry (Clemens and Inslee, 1968). The percentage of males in any of the treatments was not as high in the current experiment as in experiments that made use of synthetic androgens (Cruz and Mair, 1994; Gale et al., 1999). Even though the concentration of the saponin mixture added to the diet was much higher than the 60 mg kg⁻¹ of synthetic androgens used in most studies, the concentration of saponins actually present was lower because the mixture used is known to have only approximately 20% of these compounds (approximate content of 10% sapogenin), the remaining 80% being made up of tannins and
polyphenols (Kensil, 1996). Among the saponins themselves, there may be differences in effectiveness of the different compounds present. The highest proportion of males was found in the S700 and S700/C groups. However, the growth-promoting effect of dietary QS was pronounced at the much lower doses of 150 mg kg$^{-1}$ indicating potentially different mechanisms affecting the two actions of dietary QS.

Saponins have been previously reported to affect the release of hormones, such as LH, from the pituitary (Benie et al., 1990). LH is considered to
regulate all aspects of teleost reproduction (Suzuki et al., 1988a) and is particularly important for final oocyte maturation and ovulation (Suzuki et al., 1988b). We previously observed the inhibitory effect of dietary QS on ovulation in female tilapia having apparently normal eggs in their ovaries (Francis et al., 2001; and unpublished observations). It was, therefore, hypothesized that changes in LH levels would give an indication of the mechanism of action of QS on tilapia at the pituitary level.

We were able to demonstrate that QS promotes LH release from cultured pituitary cells of tilapia. The more purified saponin was more efficient in increasing the rate of hormone release indicating that saponins are the responsible active principles. Saponins from *Peterianthus macrocarpus* have been shown to stimulate both FSH and LH release by cultured pituitary cells of rat (Benie et al., 1990). Saponin also promoted an increase in uterine weight and blocked the estrous cycle in the luteal phase. Although there is some evidence that saponin can act through permeabilization of cell membranes, this seems at least not to be the only mode of action in tilapia pituitary cells, since, in the range of concentrations used, we found a dose-depndant response of LH to QS-P (Fig. 2). We also studied its effect on cultured cells in the presence of serum. Cells incubated in the usual BSA-containing medium released LH in a dose-dependent manner. In contrast, when cells were incubated with the same amount of saponin in the presence of 20% FCS, the LH release was significantly lower, and similar to the basal level. Since it is known that saponins have a high affinity for cholesterol and other lipids (see Fenwick et al., 1992), the serum protection may be due to saponin’s adsorption by serum lipids, suppressing its activity. This effect of serum protection on gonadotropin release from rat pituitary cells has also been seen with another saponin from *P. macrocarpus* (El IZZI et al., 1992).

The average LH values in vivo, however, did not show any trends with respect to the effect of dietary QS because of the extremely high variability within treatments. What was notable in these measurements were the different values for the 5- and 6-month old tilapia. The increase in serum LH in females was particularly pronounced. This large difference in LH levels may have been due to the fact that the tilapia entered the reproductive stage during this period. During the recrudescence and beginning of vitellogenesis stages LH levels in the plasma are very low while during final oocyte maturation LH levels are quite high (Tacon et al., 2000; Rothbard et al., 1991). A reproduction cycle in tilapia takes 21–28 days. It is very likely that during the time of sampling at 5 months, the fish were at the recrudescence stage, or too young to enter the reproduction stage, while at the age of 6 months the fish were at the stage of final oocyte maturation when LH levels are high.

Since dietary QS was shown to affect the sex ratio, it can be concluded that this change is not exerted via LH levels but rather via interference in either the FSH at the pituitary level or the steroids (probably androgens) at the gonadal level. The protective effect of the serum on the gonadotropin release in vitro may not be as strong in vivo due to the low lipid concentration in tilapia serum compared to 20% FCS.

It has become increasingly clear that the lipid environment of membrane proteins, including ion channels, transporters and receptors, plays an important role in their function (Bastiaanse et al., 1997). In biological membranes, cholesterol is organized into structural and kinetic domains or pools. Saponins may interact with the polar heads of membrane phospholipids and cholesterol through their OH groups. Moreover, their hydrophobic steroid backbone could intercalate into the hydrophobic interior of the bilayer. Both of these effects may contribute to altering the lipid environment around membrane proteins and activate specific receptors, channels or enzymes (Attele et al., 1999). The observed saponin-induced dose-dependent LH release from tilapia pituitary cells provides evidence for this mode of action. Moreover, the combination of natural GnRH and saponin, both at sub-maximal doses, did not elicit a higher response than each substance alone, suggesting that its mechanism of action may involve indirect activation of the receptor. Based on their structure, another suggested mode of action for saponins is through binding to the estradiol receptor in mammals (Martin et al., 1978) and fish (Latonnelle et al., 2000). After binding to the receptor, the saponin can elicit either agonistic or antagonistic effects in vivo and in vitro (Attele et al., 1999). The androgenic potency of QS in tilapia can be explained by antagonistic effects on the estradiol receptor.

The increasing trend in serum cholesterol content in male tilapia belonging to both saponin-fed
groups is in contrast to previous findings in which a saponin-induced decline in serum cholesterol was observed in gerbils and humans (Potter et al., 1993; Harris et al., 1997). There was also no decline in the serum cholesterol content of female tilapia in the saponin-fed groups relative to controls, but no dietary saponin-dependent trend was in evidence. Moreover, muscle cholesterol levels were not lower in the saponin groups compared to controls. The differences in serum and muscle cholesterol contents between males and females were also noteworthy.

During the breeding trials, three female tilapia belonging to the control group and one female fish belonging to the S300/C group produced fry over a 3-month period. In the S500 and S700 groups, one female each produced eggs and were observed to mouth-brood immediately after they were placed together for breeding in the breeding aquaria. But they spit out the eggs after 2 days (similar to females having unfertilized eggs). The dominant males (identifiable by their external reddish colouration) in the S150/C, S500/C and S700/C were especially aggressive and killed all other fish placed in the same aquaria. It has been previously reported that in trout fed a diet containing 500 ppm genistein (a phytoestrogen structurally similar to saponin), testicular development was accelerated in males, but spawning was delayed and gamete quality impaired leading to a low percentage of ovulating females, a lower fertilisation rate and a lower viability of fry (Bennetau-Pelissero et al., 2001). The results from the breeding experiments need to be treated as preliminary because of the low number of females available in some of the saponin-fed groups. Detailed trials, including artificial fertilization methods, need to be carried out to further study the effects of dietary QS on the reproduction of Nile tilapia.

In conclusion, dietary QS could potentially replace hazardous synthetic androgens in producing all-male populations or reducing female’s fertility if fed to tilapia at an early age, as is practised with synthetic androgen treatment. Quillaja extracts containing saponins are used commercially as flavourings and foam producers in a variety of human food preparations (Kensil, 1996) and have been classified as ‘generally recognized as safe’ (GRAS) in the US (Fenwick et al., 1992). The optimal concentration of QS for the production of all-male/infertile female populations and its mechanism of action need to be determined through further studies. This optimal dosage could then be fed to tilapia during the gonadal differentiation stage (up to 30 dpf for Oreochromis niloticus, Hines et al., 1999) of their life cycle.

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