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The effect of feed supplementation with *Quillaja* saponin (QS) on growth and metabolism of male Nile tilapia (*Oreochromis niloticus* L) during grow out.

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Abstract
A recent study by the FAO (FAO 2001) ranked tilapia as one of the fastest growing aquaculture species in the world. We previously found sex-specific effects of dietary Quillaja saponins (QS) in tilapia. Here the effects of dietary QS at a level of 300 mg/kg feed (S300) on growth and metabolism of male tilapia are reported. The tilapia grew from the initial average body weight of 72 g to a final average weight of 279 g (control) and 294 g (S300). The Metabolic Growth Rate (MGR was 10.6 ± 2.6 g for C and 11.1 ± 1.2 g for S300) and Food Conversion Ratio (FCR 0.84 ± 0.24 for C and 0.87 ± 0.09 for S300) tended to be better in the S300 group. There was no evidence of metabolic stress on the fish and the organ weights were similar in both groups. The average values of the growth parameters described above were considerably higher in the S300 group after the initial 9 weeks of the experiment (body weight of 245 ± 24 g against 227 ± 38 g in C, MGR of 12.7 ± 0.9 against 11.9 ± 1.6 in C and FCR of 1.07 ± 0.21 against 1.37 ± 0.7 in C). The differences between the groups narrowed during the last 3 weeks of the experiment. It could be seen that dietary QS was able to reduce the amount of feed needed to produce one gram gain by about 28% until 9 weeks of the experiment showing that supplementation of the diet with this substance has potential for considerably reducing feed costs.The optimal level and timing of supplementation need to be estimated through further feeding experiments.

Introduction
In recent years, tilapia (*Oreochromis niloticus* (L); Fig. 1) have become one of the most common commercially cultivated fish because they are robust and adapt readily to changing environments. According to the FAO (FAO, 2001), production of tilapia has increased by more than 13% per annum since 1984 placing it among the fastest growing aquaculture species in the world. About 1.07 million tonnes or 97% of farmed tilapia were produced in developing countries in 1999 with Asia alone producing about 82% of this amount (FAO, 2001). This statistic clearly shows the importance of tilapia as a culture fish in developing countries.
The female tilapia incubate their eggs orally. The eggs are carried in the mouth until the young hatch and for a short period thereafter. This parental care results in very high survival rates of fry which, in commercial systems, can often lead to overproduction of small fish with only a few reaching marketable size. This tends to be one of the few

disadvantages of tilapia as a culture fish. For this reason unisexual populations are preferred for culture, and fish farmers in some countries apply sex-reverse hormones (synthetic testosterone) to acheive this end. However, this practice is opposed by many consumers and others who fear the effects that the residues of these hormones may have on man and the environment. In this case a natural plant extract that has a similar effect on the reproductive organs of tilapia could well prove more acceptable.
Saponins are one group of compounds that may be suitable for this purpose since they have previously been found to alter the plasma levels of the hormones that regulate reproductive activity when added to diets for tilapia (Tamura et al., 1997). They are widely distributed secondary plant metabolites consisting of a sugar moiety linked to a triterpene or steroid with one or more carbohydrate side chains and occur in numerous forages and crops eaten by man and livestock (Price et al., 1987). Although the basic functions of saponins in plants are not fully understood, they have been shown to defend plants against attack by insects (Barr et al.,1998).
Recently, attention has focused on the anti-/ nutritional and physiological actions of saponins.When present in the diets of animals they are believed to have several negative effects. For example dietary saponins derived from different plants have been held responsible for depression of food intake, reduction in weight gain, accentuation of ruminant bloat, and photosensitization (Cheeke, 1996). They have also been found to have detergent properties, to produce stable foams in water, to show haemolytic activity and to have a bitter taste (Hostettmann & Marston, 1995), Although many saponins are toxic, not all are antinutritional and some even have positive effects in animals (Liener, 1994).For example saponins have been found to possess properties that are useful to man in many traditional herbal medicines, in human and animal nutrition and in health products. One example of a beneficial saponin is the crude extract of *Quillaja*. This extract,contains 9-10% saponin and has been used commercially in beverage production (Kensil,1996). More recently it has found application in aquaculture. Francis et al., (2001 a, b) showed that the addition *Quillaja* extracts consisting mainly of triterpenoid saponins to the diet of common carp resulted in increased growth and metabolic efficiency.
The experiment reported here was designed to test the effects of supplementation of a standard fish diet with *Quillaja* (mainly triterpeoid) saponins (QS) at a level of 300 mg/kg on predominantly male Nile tilapia (*Oreochromis nitloticus*) with respect to their growth and energy, metabolism during grow out.

Material and methods

Experimental fish
Tilapia reared at the Institute for Animal Nutrition and Aquaculture in the Tropics and Subtropics were used for the experiment. Fifteen two year old male fish with an average body mass of 72 g were selected from a large population and twelve of these were placed in chambers of a respirometer system as described by Focken et al., (1994) (Fig. 2). Three of the fish were then sacrificed for initial proximate analysis. The fish in the respiration chambers were randomly assigned to take either a control diet (C-group) or a diet containing 300 mg/kg QS (S300-group) respectively. Before start of the experiment fish were starved for 48 hrs. This was followed by an acclimatisation period of one week. During this period tilapia were fed at 9.6 $g/kg^{0.8}$/day with the control feed. At start of the experiment the animals were fed two diet viz. C and S300 at levels of

16 g/kg$^{0.8}$/day. After 12 weeks the experiment was terminated, animals were individually weighed, killed and dissected carefully to isolate and weigh the liver, intestine and gonads. After weighing the organs, they were added to the carcasses again and immediately deep frozen. Before analysis, the carcasses were autoclaved for 30 min at 120°C, homogenised, frozen and freeze dried.

Figure 1: *Oreochromis niloticus*

Figure 2: Respirometer system

Feeds and feeding regime

The two experimental diets (S300) and (C) were prepared from the same basal diet to ensure uniformity in composition. The ingredients and chemical composition of the basal diet are shown in Table 1.

Table 1. Chemical composition of diets

Ingredients and chemical composition	
Fish meal (%)	50.0
Wheat meal (%)	42.0
Sunflower oil (%)	4.0
Mineral premix (%)	2.0
Vitamins (%)	2.0
Crude Protein (N x 6.25), (%)	39.2
Petroleum ether extract (%)	9.6
Ash (%)	11.5
Gross energy, kJ g^{-1}	18.5

In Control (C), the basal diet was only ground without any addition of supplement and was put together, mixed and prepared in same manner like (S300) to insure similarity, and formed into a moist pellet. While preparing the (S300 diet), 300 mg QS (*Quillaja* saponin S-2149, Sigma Chemical Co. St Louis, Missouri USA) per kg feed were dissolved in 100 ml of distilled water and mixed thoroughly with the other ingredients before pelleting. The feed pellets in both were about 3mm in diameter and the moist in them were frozen, freeze-dried and stored in a freezer at -18°C until use. During the experiment, feeding was done individually with automatic feeders. A day's ration was spread in instalment portions to drop seven times through a funnel connected to the

respiration chamber. Feeding commenced at 07.00hrs hrs in the morning with interval of two hours between meal and last drop for the day was at 19.00 hrs. The fish were weighed at the end of every week and ration adjusted in accordance with the new body mass. In case delay in feeding was observed in a particular fish the quantity of feed provided each time to this fish was adjusted to reflect it's consumption capacity at this given time and the quantity of feeding offers adjusted so that feed wasting was minimised.

Analyses
Samples of feed and freeze dried carcasses were analysed for chemical composition according to the official and accepted methods (Naumann and Basler 1983) i.e dry matter by drying to constant weight at 105°C, crude protein as macro-Kjeldahl Nx 6.25, lipids through extraction process with petroleum ether and the gross energy by bomb calorimeter (IKA C 7000) with a benzoic acid standard.

Calculations and statistics
Each fish had its data separately calculated and the bases with which all the calculations were done are as follows: , Feed Conversion efficiency (FCE) was computed as = live weight gain [g]/dry matter of feed consumed [g], Metabolic Growth Rate (MGR) = live weight gain[g]/average metabolic live weight ($kg^{-0.8}$/ days of the experiment, Routine Metabolic Rate (RMR) = mg O_2 consumed/$kg^{0.8}$/day. Oxygen uptake in [g]x 14.85 gave the energy expenditure in kJ/g during the whole experiment (Huisman, 1976) and the Energy Apparently not Metabolised (AUE) was calculated by subtracting the energy expenditure and energy retention (ER, gross energy gain of the carcass) from the gross energy of the feed consumed. Protein Efficiency Ratio (PER) = live weight gain in [g]/ protein in feed consumed. [g] Protein Productive Value (PPV) and Apparent Lipid Conversion (ALC) were calculated as protein gain x 100/protein in feed and total lipid gain x 100 / total lipid in feed. Calculation on the following organs were Hepatosomatic Index (HSI) = fresh liver weight, Intestine Somatic Index (ISI) = fresh intestine weight and Gonads-Somatic Index (GSI) = fresh gonads Weight all divided by the fresh body weight multiplied by 100.
Statistical comparisons between the feeding groups were made using the student's test (Statistica for Windows, release 5.1 H, 1997 edition). The significance of observed differences was tested at $p < 0.05$.

Results

The tilapia grew from the initial average body weight of 72 g to a final average weight of 279 g (C) and 294 g (S300).The Metabolic Growth Rate (MGR) = 10.6 ± 2.6 g $kg^{-0.8}$ d^{-1} for C and 11.1 ± 1.2 g for S300), The Routine Metabolic Rate (RMR, mgO_2 $kg^{-0.8}$ h^{-1}) was 202.3 ± 15.5 for C and 206.0 ± 15.1 for S300. The S300 group had lower carcass, lipid and ash content (14.9 ± 1.7 % for C and 13.8 ± 2.6 % for S300) and a slightly higher PPV compared to C (Table 2).
Difference were however in EE with C lower 26.8 ± 3.8 and S300 29.2 ± 2.5, ER was lower in C 40.6 ± 10.6 and higher in S300 with 41.9 ± 4.7 and AUE significantly higher in C as compared to 20.6 ± 15.1 in S300 Table 3, and Food Conversion Ratio (FCR) 1:0.84 ± 0.24 g for C and :0.87 ± 0.09 for S300) tended to be better in the S300 group.

Table 2 Chemical composition of carcasses

	DM	CP in DM	CL in DM	CA in DM	GE in DM
Initial	21.3 ± 2.1	60.8 ± 3.1	4.8 ± 2.9	30.8 ± 3.9	15.2 ± 1.3
C	30.5 ± 0.5	50.6 ± 0.6	29.3 ± 3.9	14.9 ± 1.7	24.2 ± 0.7
S300	30.4 ± 0.5	51.1 ± 1.1	29.5 ± 3.6	13.8 ± 2.6	24.6 ± 0.8

DM = dry matter; CP = crude protein; CL = crude lipid; CA = crude ash; GE = gross energy;

Table 3 Food utilization parameters

Feed type:	FCE	PPV	ALC	PER
C	0.84 ± 0.24	38.9 ± 9.0	105 ± 3.2	2.15 ± 0.62
S300	0.87 ± 0.09	39.9 ± 3.9	106 ± 19.7	2.22 ± 0.24

FCE = food conversion efficiency; PER = Protein efficiency ratio;
PPV = Productive protein value; ALC = Aparent lipid conversion

There was no evidence of metabolic stress on the fish and the organ weights were similar in both groups hence no significant differences occurred. The growth parameters described above were considerably higher in the S300 group after the initial 9 weeks of the experiment (body weight of 245 ± 24g against 227 ± 38 g in C, MGR of 12.7 ± 0.9g $kg^{-0.8}$ d^{-1} against 11.9 ± 1.6 in C and FCR of 1.07 ± 0.21 against 1.37 ± 0.7 in C).The differences between the groups narrowed during the last 3 weeks Table 4 and Fig. 3.

Table 4. Energy budget of tilapia fed the experimental feeds.

	C	SD	S300	SD
Initial GE content of carcass (kJ)	231 ±	27.3	234 ±	32.8
Final GE content of carcass (kJ)	2077 ±	557	2183 ±	249
Routine metabolic rate RMR**	202 ±	15.5	206 ±	15.1
Energy expenditure, EE*	26.8 ±	3.8	29.2 ±	2.5
Energy retention, ER	40.6 ±	10.6	41.9 ±	4.7
Apparently unmetabolised energy AUE	32.7 ±	13.3	28.9 ±	6.3

GE = gross energy; EE ER and AUE are expressed as % of gross energy intake;
**RMR = mg 0_2 consumed /$kg^{0.8}$ / hour

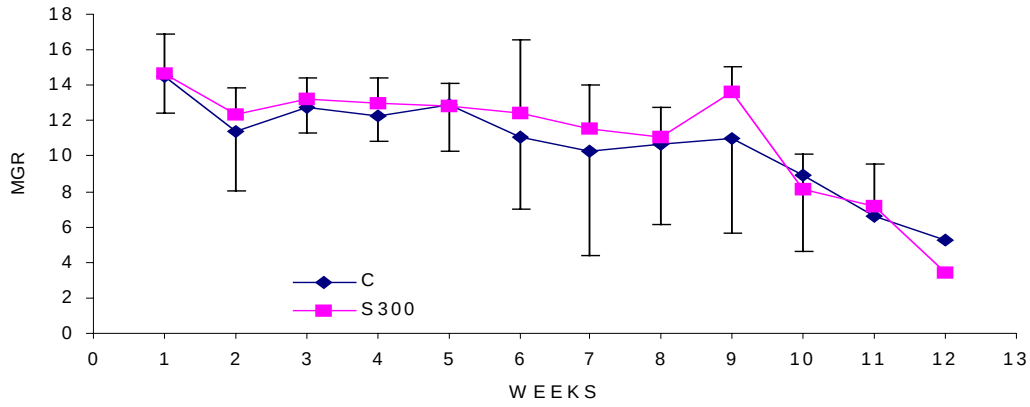

Figure 3: Metabolic growth rate (MGR) of tilapia from weeks 1 to 12

Discussion

Dietary QS had a demonstrable effect on the growth of the tilapia especially during the first 9 weeks of the experiment. It should also be noted that rearing fish in a respirometer did not inhibit their growth. The average final body mass was 279g for the control group (C) and 294g for those receiving the supplement (S300). The parameters FCE, MGR and ALC were all better in the S300 group compared to the C group .The S300 group also utilised feed energy more efficiently. Similar RMR values for both groups indicate the absence of any QS induced stress in tilapia. Up to 9 weeks, the S300 showed no inhibition of growth as a result of being kept in a respirometer. The retardation in growth observed from 10 weeks onwards could be partly attributed to a relative lack of space in the respirometers since by that time, the fish had attained an average body mass of 294g compared with only 72g at start of the experiment. Up to week 9 of the experiment, the fish receiving dietary QS needed about 28 % less feed to produce one gram of body mass gain showing that diet supplementation with QS has a potential for considerably reducing feed costs. However, the optimal level and timing of supplementation need to be ascertained through further feeding experiments. At a more general level, if farmers in the developing countries, can reduce their costs and achieve their production targets through improved feed conversion efficiency the result will be more food for a burgeoning population and a step in the direction of improved food security

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