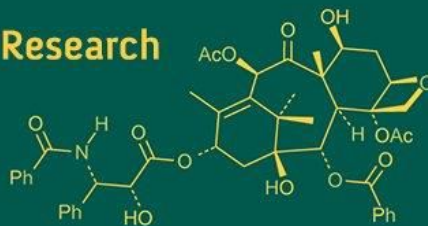
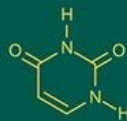


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Effects of dietary incorporation of taurine as an additive on growth and nutrient utilization of *Oreochromis mossambicus*

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Abstract

Taurine is a conditionally essential amino acid in fish nutrition. The present study addressed practical application and evaluated the impact of soybean meal diets supplemented with dietary taurine as an additive, on growth and nutrient utilization of tilapia, *Oreochromis mossambicus*, in addition to determination of maximum incorporation level of taurine in its feed. The experimental design was completely randomized with one control and four treatments. Control and each treatment had three replicates. A total number of 150 healthy *Oreochromis mossambicus* fry with an average weight of 29.6 ± 0.033 g were stocked in fifteen water tanks of 100 Litre capacity. Total five iso-nitrogenous (30% CP) iso-caloric (6% CL) feeds were prepared with taurine supplementation of 0, 0.5, 1.0, 1.5 and 2% respectively. Feed was provided ad libitum three times daily at 9:00 am, 12:00 noon and 5:00 pm. The water quality parameters like temperature, pH, dissolved oxygen (DO), ammonia were monitored on weekly basis and the growth response at the end of feeding experiment was recorded. On the experimental trial the fish fed with 1.5% supplemented taurine showed significant difference ($p < 0.05$) in all parameters like weight gain (39.23 ± 0.9), specific growth rate (0.81 ± 0.1), the nutrient utilization factors like FCR (1.54 ± 0.02), PER (2.16 ± 0.23), LRE (0.025 ± 0.004). It was concluded that the plant based feed supplemented with 1.5% of taurine results in comparatively higher growth & better nutrient utilization of *Oreochromis mossambicus*.

Keywords: Taurine, *Oreochromis mossambicus*, tilapia, growth, nutrient utilization

Introduction

Aquaculture has grown rapidly during the last few years, fuelled by the rising per capita consumption of seafood (Naylor *et al*, 2021) ^[1]. This growth was supported by a significant research effort to increase the industry's sustainability. During the last two decades, a lot of research was focused on the potential of plant-derived ingredients as an alternative to fishmeal and fish oil in aquafeed formulations. However, the dietary replacement of fishmeal with plant ingredients may result in an unbalanced supply of key nutrients. For the past decades, fishmeal (FM) has been used as the major source of protein in commercial aquafeed due to its high palatability, high digestibility, excellent amino acid profile, and numerous trace elements required for improved growth performance and feed utilization of aquaculture species. However, today it is considered both environmentally and ecologically unsustainable and there are societal and economic pressures on the aquaculture industry to find alternative proteins (Turchini, G. M *et al* 2019) ^[2]. Plant proteins are the most common replacements for fishmeal in aquafeeds. They are cost-effective and are often preferred (Gajardo, K. *et al*. 2017) ^[3]. However, even when aquafeeds high in plant protein (> 50%) are formulated to provide the required balance of amino acids and other essential nutrients (e.g. fatty acids, macro and trace minerals), the growth performance obtained is inferior to that of fish fed fishmeal-based diets. These shortcomings are often the result of plant proteins possessing anti-nutritional factors (e.g. phytate, saponins, lectins) and indigestible carbohydrates, as well as less efficient protein digestion and amino acid absorption. There has been considerable research on re-formulating aqua feeds using novel ingredients and nutritional supplements (e.g. exogenous enzymes, bioactive compounds and bioavailable trace metals) that complement plant proteins and help to meet the needs of aquaculture species.

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Taurine or 2-aminoethanesulfonic acid is a neutral b-amino acid derived from the metabolism of sulphur containing amino acids. Taurine, in addition to promoting growth, regulates the metabolism of glucose and lipids (Zhang *et al.*, 2019) [15]. Taurine synthesis ability is species-specific; thus, taurine requirements are varied among fish species. Taurine synthesis occurs in the liver, but it seems to be limited in fish, especially in marine fish, due to the low levels or absence of rate-limiting enzymes in taurine biosynthesis (El-Sayed *et al.*, 2014) [17]. Taurine is also involved in the synthesis of bile salts, which are essential for the emulsion, digestion, and absorption of dietary lipids and fat-soluble vitamins. Bile acids are synthesised from cholesterol in the liver, with cholesterol 7 α -hydroxylase acting as the rate-limiting enzyme (Hundt *et al.*, 2022) [18]. Bile acids are then conjugated with an amino acid, which in fish is mainly taurine, to form bile salts that are secreted into the bile (Hofmann *et al.*, 2010) [19]. Taurine in bile-acid synthesis has crucial importance in lipid metabolism and that is thus evident. Some studies showed an improved growth performance in carps (*Mylopharyngodon piceus* and *Ctenopharyngodon idella*), catfishes (*Clarias gariepinus* and *Pelteobagrus fulvidraco*), Nile tilapia (*Oreochromis niloticus*), and rainbow trout (*Oncorhynchus mykiss*) when taurine was included in high-or all-plant diets (Adeshina *et al.*, 2020, Gaylord *et al.*, 2006, Michelato *et al.*, 2018) [20, 21, 22]. Furthermore, taurine deficiency impaired bile pigment (biliverdin and bilirubin) production and secretion, inducing green liver syndrome in species such as red sea bream (*Pagrus major*) and yellowtail (*Seriola quinqueradiata*) (Takagi *et al.*, 2005, Takagi *et al.*, 2011, Takagi *et al.*, 2010) [23, 24, 25].

Animal proteins are rich in taurine, whereas plant proteins are taurine-deficient. Fish fed plant based ingredients require exogenous taurine. Taurine is not incorporated into proteins, nor degraded by mammalian tissues (Kuzmina *et al.*, 2010) [26]. However, it is the most abundant free amino acid in animal tissue, because it accounts for 30-50% of the entire amino acid pool, depending on the animal. Recent studies have indicated that taurine synthesis widely differs between fish species and demonstrated that it plays a key role in aquaculture and nutrition of freshwater and marine fishes.

Oreochromis mossambicus (Mozambique tilapia) is a dull colored freshwater fish which belongs to family Cichlidae. It is laterally compressed, and has a deep body with long dorsal fins, the front part of which has spines. Adults reach approximately 35 centimetres (14 in) in length and up to 1.13 kilograms (2.5 lb). It is found in many tropical and subtropical habitats around the globe. It is considered a suitable species for aquaculture for its tolerances to both crowding and relatively poor water quality and low susceptibility to diseases. They readily adapt to available food sources and efficiently utilize plant ingredients in their diet. The broad diet helps the species to survive in diverse locations. They have a mild, white flesh that is appealing to consumers. This species constitutes about 4% of the total tilapia aquaculture production worldwide. Considering the previous studies, it seems that fishes, require a supplemental taurine source when fed plant-protein-based diets to properly modulate physiological functions. The tilapia species above described was selected as a potential experimental animal to deduce results for the supplemented taurine in soybean based diets. Therefore, this study aims to evaluate the effects of dietary taurine supplementation to

evaluate growth and nutrient utilization of *Oreochromis mossambicus*.

Materials and Methods

Location of study

The study was conducted in the recirculatory wet lab of western campus in Kerala University of Fisheries and Ocean Studies, Panangad Kochi, Kerala 682506, India. Feed preparation and chemical analysis was carried out in the Nutrition and Feed Technology Laboratory, KUFOS.

Selection of fish

Tilapia (*Oreochromis mossambicus*) was selected in the present study because it is a hardy fish, accepts wide range of food and also due to its ease of availability.

Experimental setup

150 number of tilapia (*Oreochromis mossambicus*) were procured from KUFOS hatchery. The fish were disinfected with KMnO₄ solution (1ppm), and kept in 1000 Litre fibre-reinforced plastic tank for 1 week acclimatization. During acclimatization period fish were fed with commercial tilapia feed up to satiation thrice a day. After one week, 10 *Oreochromis mossambicus* fry (29.6 $\pm$ 0.33g) were stocked in each tank of 100 L capacity. Total 15 experimental tanks were used, out of which three tanks were for control and other twelve were equally allocated under four dietary treatments. The experimental tanks were provided with sponge filters and aeration facility. The excreta was removed daily at morning and 50% water was exchanged at weekly basis to avoid deterioration in the quality of water due to accumulated organic matter and metabolites. Prior to initiation of the study the tanks were cleaned, disinfected and dried. The tanks were covered with wide meshed nylon nets to prevent extraneous matter falling into tanks and also to prevent fish from jumping out of tanks. The volume of water in the each tank was 70 liters. This level was maintained throughout the experimental period.

Feeding and Feed Formulation

Fishes were fed ad libitum thrice at 9:00 am, 12:00 and 5:00 pm daily. Before commencement of the experiment, the fishes were acclimatized to the experimental diets for a week. Left over feed of the previous day, if any, was removed prior to feeding. The feed was formulated from the ingredients procured from local market, as follows:

Raw materials

The ingredients used for the preparation of the test diets were soybean meal, groundnut oilcake, maize, rice bran, vegetable oil, vitamin and mineral mixture, salt, cellulose, carboxymethylcellulose and taurine. All the ingredients were procured from local market, near Kochi, Kerala. Ingredients were powdered and sieved to obtain uniform particle size (less than 200 micron).

The ingredients and their proportion used in the formulation of feeds is given in Table, below:

The diets were designated as Control, Treatment 1, Treatment 2, Treatment 3 and Treatment 4. The control diet was having all the ingredients except taurine. Diet T₁ (Treatment 1) contained 0.5% taurine, diet T₂ (Treatment 2) 1.0%, diet T₃ (Treatment 3) 1.5% and diet T₄ (Treatment 4) 2% taurine.

The ingredients and their proportion used in the formulation of experimental diets.					
Ingredient	C	T ₁	T ₂	T ₃	T ₄
Soybean	40	40	40	40	40
Groundnut oil cake	23	23	23	23	23
Maize	14	14	14	14	14
Rice bran	14	14	14	14	14
Vegetable oil	2	2	2	2	2
Vitamin Mineral Mixture, Supplevit-M*	1	1	1	1	1
Salt	1	1	1	1	1
Cellulose	4	3.5	3	2.5	2
Carboxymethyl cellulose	1	1	1	1	1
Taurine	0	0.5	1.0	1.5	2
Total	100	100	100	100	100

Composition of Supplevit-M (Vitamin Mineral mix); Quantity in each 1.25Kg			
Calcium	225 g	Zinc	9.60 g
Phosphorus	127.5 g	Iron	1.50 g
Magnesium	6 g	Copper	4.20 g
Manganese	1.50 g	Cobalt	150 mg
Vitamin A	700000 IU	Selenium	10 mg
Vitamin D3	7000 IU	Sulphur	7.20 g
Vitamin E	250 mg	Potassium	100 mg
Nicotinamide	1000 mg	Sodium	6 mg
Iodine	325 mg	Chromium	78 mg

The ingredients and their proportion used in the formulation of experimental diets.					
Ingredient	C	T ₁	T ₂	T ₃	T ₄
Soybean	40	40	40	40	40
Groundnut oil cake	23	23	23	23	23
Maize	14	14	14	14	14
Rice bran	14	14	14	14	14
Vegetable oil	2	2	2	2	2
Vitamin Mineral Mixture, Supplevit-M*	1	1	1	1	1
Salt	1	1	1	1	1
Cellulose	4	3.5	3	2.5	2
Carboxymethyl cellulose	1	1	1	1	1
Taurine	0	0.5	1.0	1.5	2
Total	100	100	100	100	100

Composition of Supplevit-M (Vitamin Mineral mix); Quantity in each 1.25 Kg			
Calcium	225 g	Zinc	9.60 g
Phosphorus	127.5 g	Iron	1.50 g
Magnesium	6 g	Copper	4.20 g
Manganese	1.50 g	Cobalt	150 mg
Vitamin A	700000 IU	Selenium	10 mg
Vitamin D3	7000 IU	Sulphur	7.20 g
Vitamin E	250 mg	Potassium	100 mg
Nicotinamide	1000 mg	Sodium	6 mg
Iodine	325 mg	Chromium	78 mg

Supplevit-M: Zydus AH, Ahmadabad.

Formulation and processing of test diets

The ingredient proportion of the diet is given in Table above. The overall protein content of the feed was kept 30%. The quantities of the ingredients for each diet were weighed out and mixed thoroughly to ensure uniform distribution. For the feed ingredient mix, just enough quantity of water was added to make a smooth dough. The dough was cooked under pressure for 30 minutes. To the cooled dough vitamin mineral mix @ 1% was added and mixed well to ensure uniform distribution. The dough was then pelletised using a hand pelletiser with 2mm of die. The pellets were dried in a hot oven to a moisture content of less

than 10%. The dried pellets were cooled and then crumbled to adequate size to be taken by the tilapia fry, packed in air tight plastic bags and used for feeding.

Growth evaluation

Fish were sampled at 10 days intervals, in the morning, before feeding. Fish were caught through scoop net and individual as well as mass weight of fish was recorded. At end of the feeding experiment, the individual final weight, length and total biomass in each tank were recorded. Fishes were sampled at 10 days intervals, to record the increments in weight of fish fed different test diets. And other parameters were accordingly collected and recorded for further usage in the overall data. After completing the feeding trial of 45 days, fishes were taken for parameters of growth & nutrient utilization. On termination of the experiment, surviving fishes were collected for further statistical analysis and the tanks were dewatered.

Determination of growth & nutrient utilization

Different parameters were recorded like weight gain, weight gain percentage, specific growth rate (SGR), condition factor (CF), Hepato-somatic index (HSI), Viscerasomatic index (VSI), Survival (%), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Protein gain, Lipid gain, Protein retention efficiency (PRE) and Lipid retention efficiency (LRE) were calculated based on the acquired data during experimental period to assess the impact of dietary taurine on growth and nutrient utilization of *Oreochromis mossambicus*.

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Weight gain (\%)} = \left[\frac{(\text{Weight gain (g)}) \times (\text{Initial weight (g)}^{-1})}{1} \right] \times 100$$

$$\text{SGR} = 100 \times \left[\frac{\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})}{\text{days of experiment}^{-1}} \right]$$

$$\text{Condition Factor (CF)} = 100 \times \left[\frac{\text{Final body weight (g)}}{\text{Total length}^{-3} \text{ (cm)}} \right]$$

$$\text{Hepato-somatic index (HSI)} = 100 \times \left[\frac{\text{weight of liver (g)}}{\text{weight of fish (g)}} \right]^{-1}$$

$$\text{Viscera somatic index (VSI)} = 100 \times \left[\frac{\text{weight of viscera (g)}}{\text{weight of fish (g)}} \right]^{-1}$$

$$\text{Survival (\%)} = \left(\frac{\text{Number of fish on final sampling} \times \text{Number of fish at initial stocking}^{-1}}{1} \right) \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (g)}}{\text{wet biomass gain (g)}}^{-1}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Biomass gain (g)}}{\text{Crude protein fed (g)}}^{-1}$$

$$\text{Protein gain} = \left(\frac{\text{Final biomass} \times \text{Final crude protein content}}{\text{Initial biomass} \times \text{Initial crude protein content}} \right)$$

$$\text{Lipid gain} = \left(\frac{\text{Final biomass} \times \text{Final crude lipid content}}{\text{Initial biomass} \times \text{Initial crude lipid content}} \right)$$

$$\text{Protein retention efficiency (PRE)} = 100 \times \left[\frac{\text{Protein gain (g)}}{\text{Total crude protein fed (g)}} \right]^{-1}$$

Lipid retention efficiency (LRE) = $100 \times [\text{lipid gain (g)}] \times [\text{Total crude lipid fed (g)}]^{-1}$.

Water sampling

The water samples were collected during 8:00 to 9:00 am at weekly intervals and various parameters viz., pH, temperature, dissolved oxygen and ammonia were determined.

Body composition

At the beginning 40 fish were sacrificed and kept for analysis. At the end of the experiment during final sampling, 3 fish were killed from each tank and stored for body composition analysis.

Sampling for proximate composition of feed

After the feed preparation, 50 g of dried feed from each treatment was kept for proximate composition.

Physico-chemical analysis of water

Water temperature

The water temperature was measured by using a 0.0 to 50.0 °C graduated (0.1 °C) mercury thermometer and values expressed in °C.

pH

The water samples were brought to laboratory and pH of water was measured potentiometrically with the help of pH meter.

Dissolved oxygen

The dissolved oxygen content of water was measured using dissolved oxygen meter (Thermo Electron Corporation, Orion 3 star DO portable).

Ammonia

Total ammonia was assessed by using the phenol hypochlorite spectrophotometric method.

Proximate composition

The fish and feeds were analysed for initial and final proximate composition by using AOAC (1995) methodology. Crude protein content was determined by Kjeldahl method. Crude fat was determined by Soxhlet extraction. The ash content of diets and fish tissues was determined by dry ashing in a porcelain crucible and placed in a muffle furnace at 550 °C for 4hrs.

Statistical analysis

All the data are expressed in mean $\pm$ standard error (Mean $\pm$ SE). The data were compared using a one-way analysis of variance (ANOVA), and differences between means were tested for significance using Duncan multiple range test. The significance level was set at $p < 0.05$. Statistical analyses were performed with the software package SPSS Version 16.0.

Results

The efficiency of experimental diets incorporated with taurine was performed on the culture of *Oreochromis mossambicus* for 45 days in the wet laboratory condition. The feed without taurine was set as the control diet. Detailed results of this study on the various parameters viz., water quality of the rearing tanks, proximate composition of fish, survivability, growth performance, nutrient utilisation, liver antioxidation status and enzyme activity of *O. mossambicus* were recorded during the period of investigation. The results are presented under the following headings:

Growth pattern & somatic attributes

The growth of Tilapia was recorded by taking the weight of whole biomass at the end of feeding experiment and between the days, weekly. All the treatments showed an increasing growth trend over the culture period. The growth determination factors such as initial weight, final weight, weight gain, weight gain%, SGR and survivability of *Oreochromis mossambicus* fed with different feeds having taurine concentration of 0%, 0.5%, 1.0%, 1.5% and 2.0% respectively over a period of 45 days is represented in Table below:

Somatic attributes of *Oreochromis mossambicus* fed with experimental diets.

Days	Control	0.5% Taurine (T ₁)	1.0% Taurine (T ₂)	1.5% Taurine (T ₃)	2.0% Taurine (T ₄)
Initial weight	29.33 $\pm$ 0.33	30.33 $\pm$ 0.33	29.33 $\pm$ 0.33	29.67 $\pm$ 0.33	29.67 $\pm$ 0.33
Final weight	52.50 $\pm$ 0.51	58.97 $\pm$ 0.56	62.30 $\pm$ 0.57	68.90 $\pm$ 0.31	68.87 $\pm$ 0.28
Weight gain	23.17 $\pm$ 0.38 ^a	28.63 $\pm$ 0.38 ^b	32.97 $\pm$ 0.90 ^c	39.23 $\pm$ 0.07 ^d	39.2 $\pm$ 0.06 ^d
Wt. gain%	79 $\pm$ 1.54 ^a	94.41 $\pm$ 1.46 ^b	112.48 $\pm$ 4.27 ^c	132.28 $\pm$ 1.63 ^d	132.17 $\pm$ 1.68 ^d
SGR	0.56 $\pm$ 0.01 ^a	0.64 $\pm$ 0.01 ^b	0.73 $\pm$ 0.02 ^c	0.81 $\pm$ 0.01 ^d	0.81 $\pm$ 0.01 ^d
VSI	10.4 $\pm$ 0.02 ^a	10.08 $\pm$ 0.02 ^a	10.14 $\pm$ 0.02 ^b	10.18 $\pm$ 0.02 ^b	10.17 $\pm$ 0.02 ^b
HIS	0.60 $\pm$ 0.01 ^a	0.69 $\pm$ 0.00 ^b	0.78 $\pm$ 0.01 ^c	1.02 $\pm$ 0.01 ^d	1.0 $\pm$ 0.01 ^d
Condition Factor	1.58 $\pm$ 0.02 ^a	1.59 $\pm$ 0.01 ^a	1.64 $\pm$ 0.00 ^b	1.70 $\pm$ 0.01 ^c	1.71 $\pm$ 0.01 ^c
Survivability	100	100	100	100	100

Final weight

The final weight of Tilapia at different dietary treatments ranged between 52.8 to 69.1g. The result showed significant difference ($p < 0.05$) among the treatments. The group fed with 1.5% and 2% taurine exhibited significantly highest final weight.

Weight gain

Significantly highest value of weight gain (39.3 g) was exhibited by the group fed with 1.5% and 2% taurine. There

was significant difference ($p < 0.05$) among the treatments. The value of weight gain ranged between 22.5-39.3g.

Weight gain percentage

The result showed significant difference ($p < 0.05$) among the treatments with highest weight gain% in the group fed with 1.5% & 2% taurine. The other three groups viz., Control, T₁ & T₂ exhibited gradual increase in weight gain percentage respectively.

Specific growth rate (SGR)

The significantly highest ($p<0.05$) SGR was recorded in the group fed with 1.5% and 2% taurine. However, the rest three groups viz., Control, T₁ & T₂ exhibited significantly lower but gradual increase in SGR.

Viscero-somatic Index (VSI)

The viscero-somatic index (VSI) shows significant difference ($p>0.05$). Treatment 2, 3 and 4 showed similar values. However control and treatment 1 showed significantly lower but almost matching values.

Hepato-somatic Index (HSI)

The hepato-somatic index (HSI) shows significant difference ($p>0.05$). Treatment 3 and treatment 4 showed significantly higher but similar values. However control,

treatment 1 and treatment 2 showed significant gradual increase but lower values.

Condition Factor (CF)

The Condition Factor (CF) shows significant difference ($p>0.05$). Treatment 2, 3 and 4 showed similar values. However control and treatment 1 showed significantly lower but almost matching values.

Nutrient utilization

The nutrient utilisation was expressed with FCR, FER, PER, PRE of *Oreochromis mossambicus* fed diet incorporated with taurine at levels of 0%, 0.5%, 1.0%, 1.5% and 2% over a period of 45 days in the experimental tanks is presented below.

The nutrient utilization of Tilapia fed with the experimental diets.

		0.5%	1.0%	1.5%	2%	
Nutrient	Control	Taurine	Taurine	Taurine	Taurine	P
Utilisation		(T ₁)	(T ₂)	(T ₃)	(T ₄)	Value
FCR	2.69±0.05 ^e	2.29±0.05 ^d	1.95±0.02 ^c	1.54±0.02 ^a	1.66±0.01 ^b	$p<0.05$
FER	0.37±0.01 ^d	0.44±0.01 ^c	0.51±0.01 ^b	0.65±0.01 ^a	0.60±0 ^a	$p>0.05$
PER	1.24±0.02 ^d	1.46±0.03 ^c	1.69±0.04 ^b	2.16±0.03 ^a	2.01±0.01 ^a	$p<0.05$
PRE	0.192±0.001 ^e	0.226±0.001 ^d	0.314±0.007 ^c	0.461±0.003 ^a	0.420±0 ^b	$p>0.05$
LRE	0.001±0.0001 ^d	0.008±0.0001 ^c	0.014±0.002 ^b	0.023±0.005 ^a	0.025±0.004 ^a	$p>0.05$

Feed conversion ratio (FCR)

Among the different treatments, except treatment 2 other three groups showed lower FCR. The significant difference ($p<0.05$) is presented in Table above. The lowest value of FCR was observed in the treatment 3 in which taurine concentration was 1.5%. The value of FCR ranged between 1.50 to 2.77.

Protein efficiency ratio (PER)

The significantly higher PER was recorded in treatment 3 and 4 fed with 1.5% and 2% taurine respectively. The PER value ranges from 0.19 to 0.42. There was significant ($p<0.05$) difference between the treatments.

Protein retention efficiency (PRE)

Higher protein retention efficiency (PRE) was shown in treatment 3 fed with 1.5% taurine. There was significant ($p>0.05$) difference between the treatments.

Conclusion

Plant protein sources are mostly deficient with two limiting amino acids such as cysteine and methionine (Espe *et al.*, 2008) which are the main precursors of taurine synthesis thus taurine is not found in plant ingredients. The inclusion of high levels of plant protein in fish feeds results in the lower performances in comparison to fish meal based feeds, possibly due to the inability, or low ability, of the fish to synthesize taurine. Taurine supplementation (1.5%) results in comparatively higher growth & nutrient utilization of *Oreochromis mossambicus*, fed plant based feed.

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