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ALTERNATIVE PROTEIN SOURCES AS A REPLACEMENT OF FISH MEAL IN TILAPIA FEEDS

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ABSTRACT

A 32 day trial with Genetically Male Tilapia (Oreochromis niloticus) was conducted to evaluate several commercially available ingredients polymeal, concentrated peaseed meal, and Marine Protein Substitution (MPS) as alternative protein sources instead of fishmeal. Four experimental diets were formulated to contain the same levels of protein (40%) and lipid (10%) and each ingredient was the only protein source. The feeds were fed to triplicate groups of tilapia fingerlings of initially 5.7g. During the course of the trial, tilapias were fed manually to apparent satiation up to four times daily. The highest feed intake and growth performance of tilapia were obtained with tilapia fed the fishmeal, followed by the group fed polymeal and MPS where both diets achieved the same results regarding feed intake and growth rates. However, the lowest feed intake and growth rate were found in tilapia fed the peaseed meal concentrate. The other observation from the present study is fishmeal, polymeal and MPS meal had almost the same Food Conversion Ratio (FCR) that ranged between1.22 and 1.25. Tilapia fed the peaseed meal on the other hand had the best FCR of 0.86 and the highest protein efficiency ratio of 38.5%. This suggests, peaseed meal as a protein source was used effectively by tilapia, however, feed consumption was low and thus the overall growth. Therefore, it can be concluded that fishmeal could be replaced by polymeal or MPS meal without adverse effects, however, the low palatability of peaseed meal would require an additional attractant in the feed.

KEYWORDS: *Fishmeal, Polymeal, Peaseed Meal, MPS Meal, Growth Performance, Feed Intake*

INTRODUCTION

Tilapia is classified as an omnivorous fish and it has been successfully grown on a low-cost commercial diet. The optimum growth of tilapia requires different diet formulations that include proteins (amino acids), lipids (fatty acids), energy sources (lipids, protein, and carbohydrates) and vitamins. To achieve growth in fish, the requirement for the deposition of new body components has to be satisfied, which in fish consist mainly of protein and lipids (Lupatsch et al. 2003). Thus, feed must supply protein and lipid to build new tissue, but also energy is important for protein and lipid synthesis (Lupatsch 2009; Lupatsch et al. 2003). Therefore, total requirements of protein and energy for growing tilapia are the sum of demands for growth and maintenance. The requirement for maintenance is depended on the fish size and the water temperature, while the requirement for growth is depended on the composition of the weight gain (Lupatsch 2008; Lupatsch et al. 2003). Protein is – besides energy – one of the essential components in fish nutrition, as it provides the fish with the essential amino acids which are important parts in order to build body proteins (Yang et al. 2002). Fish digest the protein to release free amino acids that are important to build vital tissue of fish's bodies. Tilapia – like all fish species - needs ten essential amino acids for good growth performance, such as valine, leucine, lysine, methionine, isoleucine, histidine, phenylalanine, threonine, arginine, and tryptophan (Halver & Hardy 2002). According to Abdel-Tawwab et al. (2010), the optimal dietary protein level for best growth depends on fish size. Thus feeding fry Nile tilapia 45 % protein diets resulted in a high growth performance, and 25 % of protein diets caused a poor growth of fry Nile tilapia. However, for the larger tilapia, a lower protein feed was sufficient. Therefore, protein gained from tilapia increased as a result of increasing protein levels in the diet for both fry and adults tilapia fish. In contrast, protein retention efficiency in the fish related negatively with increasing the level of protein to energy intake. At protein intake above the requirements, the protein could be used as an energy source to deposit the lipid instead of its main usage for protein deposition, thus protein efficiency in fish would be reduced for its essential role of protein deposition (Lupatsch et al. 2003). Requirements of sparing protein for growth with rising dietary lipid as a source of energy are an important condition for farmed fish. According to De Silva et al. (1991), the occurrence of protein sparing in farmed tilapia was associated with increasing the dietary lipid up to 18 %. Lipids are a source of fatty acids and energy. Fish are able to store lipids in their bodies thus an improper dietary protein to energy ratio might affect the body composition. The highest level of body lipid content for hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* was when they were fed a 20 % lipid diet, followed by hybrid tilapia fed on 10-15 % lipid diets. The lowest level of body lipid content recorded in tilapia fed 5 % of lipid diet, therefore the best level of dietary lipid for optimum growth of hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* was 12 % (Chou & Shiau 1996).

Consequently, development of feed with proper protein to energy ratios is essential for cost-effective feed formulation (Lupatsch 2008). Dietary protein requirement varies between carnivorous and omnivorous fish species, as omnivores and herbivores require 24-32% of dietary protein, whereas carnivores require 45-50% of the protein in their diet. This might be related to that, carnivorous species consume less food than omnivorous species, thus including higher levels of protein in the feed of carnivores will be suitable to achieve the optimum growth (Lupatsch 2009). Tilapia diets similar to other animal feeds are made from different feed ingredients to satisfy the nutrient requirements; protein and energy needs are expressed in terms of the demand per fish weight gain and body mass (Lupatsch 2009). So, feed formulations that are based on daily requirements for protein and energy can be formulated according to the anticipated growth (Lupatsch & Kissil 2005; Lupatsch et al. 2003).

This experiment was to determine if the fishmeal can be totally replaced with peaseed meal, polymeal, or Marine Protein Substituted (MPS) (Trademark ingredient of Dragon feed Ltd) meal in the diet of tilapia. This was investigated using four different diets, as the single source of protein. Fishmeal and peaseed are ingredients commonly available, whereas 'polymeal' and 'Marine Protein Substitute' are trademark ingredients produced by the local feed manufacturing Dragon Feeds Ltd, Port Talbot. The aim of this investigation was to compare the growth performance of tilapia fed the four different diets. This investigation will provide information about the potential use of peaseed meal, polymeal, and MPS meal for farmed tilapia.

MATERIALS AND METHODS

Fish and Experimental System

Genetically Male Tilapia (GMT) were obtained as swim-up fry from Fishgen Ltd. Fry was reared at CSAR for 2 months on a commercial fish feed containing approximately 45% protein and 13% lipid until they reached the appropriate size. Prior to the trial, tilapia juveniles were hand-graded into three size groups. The actual trial was carried out using 180 fish of the medium size corresponding to ~ 5.7 g/fish.

The trial was set up indoors as part of the freshwater recirculation system using 12 tanks (water volume of 20.4 liters in each tank). The recirculation system was operated to clean and filter the water for recycling back through the fish tanks. The water from tilapia tanks first passed through the sand filter which directs the water to a packed aerated biological reactor (PABR). In this phase, the water was treated by two species of bacteria, *Nirosomonas* and *Nitrobacter* to convert the ammonia to nitrite, and then convert the nitrite to nitrate. The water also was treated to pH dosing, where the sodium hydroxide solution was added to increase the pH. Before returning the water to the fish tanks, it was passed through a UV treatment to sterilize the water by using germicidal lamps.

During the trial, the 12 tanks were covered by a plastic mesh to prevent the fish from escaping. The water flow could be adjusted for each tank separately and the flow rate was measured to be 2L/ min. In addition, the following measurements were taken once a week by the staff from CSAR as a part of the center's routine systems and water chemistry checks: temperature, salinity, pH, Alkalinity, ammonia, nitrite and nitrate. Water temperature was kept constant at 26.5-27º C using a thermostatically controlled water heater. The system was back washed once a week and new water was added to the main tank to make up for the water loss due to cleaning tanks leftover feed and waste materials.

Diet Preparation

Four experimental diets were formulated using commercial ingredients such as fishmeal, polymeal, peaseed meal concentrates and 'Marine Protein Substitute' (MPS) as the single source of protein. Fishmeal and peaseed are ingredients commonly available, whereas 'polymeal' and 'Marine Protein Substitute' are trademark ingredients produced by the local feed manufacturing Dragon Feeds Ltd, Port Talbot. According to Dragon Feeds Ltd MPS meal is a soybean derived product supplemented with amino acids. Polymeal as well is based on a soya product supplemented with amino acids, but also including marine polychaete worms (grown and harvested by Dragon Feeds Ltd) and seaweed. The composition of the ingredients used in this trial is shown in Table 1.

Diets were formulated to contain 40% crude protein and 10% lipids (Table 2). Dry ingredients for each diet were mixed properly before the rapeseed oil was added. Then, a doughy mixture was achieved by adding hot water with the help of a Kenwood kitchen hand mixer. This dough was extruded through a meat grinder with 2.4 mm diameter orifice plate. The resulting spaghetti-like strands were dried in an oven for 24 hrs at 45° C.

The dry mixture was afterward broken up by a kitchen blender, resulting in pellets of approximately 5mm in length and 2.4mm in diameter. Preliminary tests ensured that pellets were easily taken by tilapia, and kept their shape in the water for up to 24 hrs.

Feeding and Growth Trial

The four experimental diets were offered to triplicate groups of tilapia fingerlings. From the hand graded homogenous fish, groups of 15 tilapia each were batch weighed and successively stocked into 12 tanks. Also, about 15 fish were sampled and stored in the freezer providing an initial sample for subsequent analyses. After the transfer, fish were immediately switched to their new diets, which were accepted without any problems.

During the duration of the trial, which lasted for 32 days, tilapia was fed manually to apparent satiation up to four times daily. At each feeding cycle, new pellets were only added when all the feed was consumed from the feeding before. The following morning any leftover pellets were removed and counted. A sub-sample of pellets had been taken from each diet to calculate the approximate weight of each pellet. Thus the total amount of leftover feed was monitored for each tank by recording the number of leftover pellets and subtracting from the feed offered.

Daily routine besides feeding included cleaning the tanks in the morning using a plastic tube siphon to remove feces and uneaten pellets, before adding any new feed. Any dead fish were also removed and recorded.

Tilapia was batch weighed approximately every two weeks to monitor the growth. The recording of the weight and the number of fish every two weeks is needed for calculations such as a feed conversion ratio, weight gain, and specific growth rate.

Sample Preparation and Chemical Analysis

Tilapia whole body at the start and the end of the trial and the experimental diets were sampled for subsequent analysis to determine the dry matter, ash, energy and protein content.

Whole tilapia from each tank were sacrificed and frozen at 4° C. Prior to analyses, they were blended to a homogeneous mince using a meat grinder with a 4 mm diameter orifice plate. A sub-sample of this mince from each tank was taken and stored for estimation of dry matter which was determined after drying in the oven at 105°C for 24 hrs. The remaining fish homogenate was dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace for 12 hrs at 550ºC. A Parr bomb calorimeter was used to calculate the gross energy content, this method measures energy content by combustion under an atmosphere of compressed oxygen with benzoic acid as a standard. The Kjeldahl technique was used to measure crude protein. In this technique, the nitrogen (N) content is determined and multiplied by 6.25. This value is derived from the assumption that all the N comes from protein and that protein contains 16 % N. Crude lipid was measured after chloroform-methanol 2 : 1 extraction. Samples were homogenized with a high speed homogenizer for 5 min and lipid was determined gravimetrically after separation and vacuum drying.

Statistical Analysis

For the statistical analysis of this study, all data collected during the trial such as weight gain, feed intake and feed conversion ratio were tested for normality by using K-S test. Then, these data were tested for significances using one way ANOVA Post Hoc (Tukey Test).

RESULTS

Growth Performance and Feed Intake of Tilapia

Results of the actual feed analyses as presented in Table 3 confirm the intended crude protein (415 to 425 g) and lipid content (92 to 102 g) per kg feed.

Significantly highest weight gain of 0.80g per day was obtained by tilapia fed the fishmeal diet (Diet1) followed by tilapia fed the polymeal (Diet 2) and marine protein substitute (Diet 4) which was 0.66g /fish/ day, whereas significantly lowest growth performance of just 0.24g /fish/ day could be observed in tilapia fed the peaseed meal (Diet3) ($F=67.708$, $P< 0.05$) (Table 4).

After 11 days of trial, one could detect already a tendency of difference in growth, by day 11 the body weight of tilapia fed Diet 3 was lower than the other three groups. By this stage, the body weight of tilapia groups fed on Diet1, Diet2, and Diet4 was similar. By the final day (32), tilapia fed Diet 1 showed the largest body weight of about 31.4 g (Figure 1).

Significantly highest feed intake of 1g per fish per day was obtained in tilapia fed the fish meal (Diet 1), whereas the significantly lowest value of 0.21g was observed in tilapia fed the peaseed meal (Diet3). ($F=66.571$, $p<0.05$) (Table 4).

Daily feed ration (%) presented for each weighing period of the trial can be seen in Figure 2. In general, it can be said that feed intake calculated as a percentage of biomass decreased over the trial period when fish are getting larger. But it can be observed as well, that from the start of the trial % daily feed intake of tilapia fed Diet 3 was the lowest of the four diets whereas the % feed intake of tilapia fed Diet 1 was the highest during the early stages and in the overall trial period.

Tilapia fed peaseed meal (Diet 3) had the lowest FCR. Interestingly, values of FCR for tilapia fed on Diet 1, Diet 2 and Diet 4 were similar among each other, however, all were significantly different to FCR of tilapia fed Diet 3 ($F=44.241$, $P<0.05$) (Table 4).

The survival rate throughout the experiment was varied but not related to treatment. Diet 2 showed the highest survival rate (97.8 % ± 3.8), and Diet1 the lowest (71.1 % ± 23.4).

Whole Body Composition of Tilapia

According to the information in this table, it can be seen that, the lowest value of dry matter content was found in tilapia fed Diet3, as well as lipid and energy content, whereas highest dry matter, lipid, and energy content were found in fish fed the fish meal feed (Diet 1). Tilapia fed Diet 2 and Diet 4 obtained intermediate values between Diet 1 and Diet 3 regarding dry matter, lipid and energy content.

Protein and ash content were similar for all treatments, with the exception of tilapia fed Diet 3 where the lowest protein content of 143 mg / g fish and highest ash content of 47 mg / g fish were found ($F=11.844$, $P<0.05$)(Table 5).

Efficiency of Energy and Protein Retention

Tilapia fed Diet1 were able to consume the highest amount of gross energy (17.77kJ/ tilapia/ day), compared to tilapia fed Diet3 which had the lowest intake of energy (3.92 kJ/tilapia/day) as this correlates with feed intake. Thus as well the energy gained by tilapia fed Diet1 was the highest compared to fish fed the other diets. The lowest energy gain was found in tilapia fed Diet 3, again corresponding with overall weight gain. Statistical analysis showed that the differences among energy gains were found to be significant ($F=84.683$, $P<0.05$). However, regarding the efficiency with which energy was retained as growth (Table 6), no significant differences were found in tilapia fed the four diets ($F=1,528$, $P>0.05$) (Table 6).

Also, tilapia fed Diet1 consumed the highest amount of protein (0.426 g/tilapia/day) compared to those fed Diet3 which had the lowest amount of protein intake (0.086 g/tilapia/day). As a consequence, tilapia fed Diet1 was able to gain the highest amount of protein (0.13g/tilapia/day) compared to those fed Diet3 (0.03g/tilapia/day). Statistical analysis showed that the differences of protein gained were found to be significant ($F=130.027$, $P<0.05$). In addition, regarding the protein retention efficiency significant differences were found ($F=8.645$, $P<0.05$). Interestingly, the highest protein retention efficiency (38.5%) was found in fish fed Diet 3 compared to those fed Diet 1, 2 and 4 (Table 7).

DISCUSSIONS

In the present investigation, tilapia showed a very satisfactory growth, growing from 5.7g to around 31.4g in 32 days. Tilapia fed the fishmeal diet showed the best growth performance, with an average daily weight as high as 0.80g, while tilapia fed Diet 3 peaseed meal showed the lowest weight gain of 0.24g less than half of that (Table 4).

Feed intake is a very important factor in determining results from a dietary study and it is obvious that, the pattern of growth in the present study was depending on the amount of feed consumed by tilapia. However the amount of feed consumed differed according to each diet, and so did the growth performance (Figure 1).

Results suggest that the low feed intake of tilapia fed the peaseed meal was the reason for the low growth rate. Tilapia fed the peaseed meal consumed only 2.38% compared to tilapia fed other diets. In all instances, feed intake was directly associated with weight gain of tilapia (Figure 2). The results in the present study are not supported by investigations involving peaseed meals where feed intake is either unchanged or increased compared to the control fishmeal diets. According to Øverland et al. (2009) there were no differences in feed intake or weight gain of Atlantic salmon when fed diets containing fishmeal and soybean compared to diets containing 200g/kg pea protein concentrate with either 50% or 35% of crude protein. There were also no significant differences in feed intake of gilthead sea bream *Sparus aurata* when fed 30%, 60% and 90% of fishmeal substituted with diet included pea protein concentration and rice protein concentration (Sanchez-Lozano et al. 2009). The pattern of growth of tilapia fed peaseed meal in the present study was dependent on the amount of peaseed meal consumed by tilapia. According to Figure1 the body weight of tilapia fed the fishmeal diet increased dramatically. In contrast, the body weight of tilapia fed Diet3 peaseed meal was depressed. This difference was found to be significant over 32 days. This result was supported by the findings of El-Saidy & Saad (2008) who reported that Nile tilapia *O. niloticus* fed diets replacing 100% of fishmeal with cow peaseed meal obtained the lowest growth rate compared to the fishmeal only diet. However, the growth of *O. niloticus* fed on 25% and 50% inclusion of peaseed meal was not different from the growth of fishmeal diet. The present study agrees the finding of this investigation as high inclusion levels of pea protein in tilapia's diet reduced the growth of tilapia.

Besides palatability problems with some plant ingredients, one of the factors for reduced growth could be the amino acid imbalance. According to Schulz et al. (2007), pea protein isolate could replace 30 % of fish meal protein in the diet for juveniles tilapia without negative effects on fish growth response, but that higher replacement levels by pea protein caused a reduction in the growth performance. They related this result to the lysine and methionine deficiency in peaseed diet in comparison to essential amino acids requirement for Nile tilapia (Santiago & Lovell 1988).

Many studies were focused on improving peaseed as an energy and protein source in fish feeds. Davies & Gouveia (2008) found that the ingredient quality of raw peaseed meal can be improved by dry heat treatment, in particular at 180 ºC for 30 minutes to reduce trypsin inhibitor level in peaseed diet which had led to improve feed utilization and the growth performance for African catfish. Also, a dietary level of 20% of air-classified pea protein did not reduce the palatability, feed intake and growth performance of rainbow trout (Thiessen et al. 2003a). In other investigation by Thiessen et al. (2003b) who compared the growth and digestibility of rainbow trout fed diets where fishmeal was substituted by peas processed by different methods raw/de-hulled peas, extruded/de-hulled peas and autoclaved air-classified peas protein. Starch digestibility of fish fed extrusion or autoclaving was increased by 41-75%, this had led to an increase of the energy digestibility however, protein digestibility stayed unaffected. Whereas, pea protein was highly digestible 91.4% when rainbow trout fed raw/de-hulled peas. At CSAR, apparent digestibility coefficients of peaseed meal were tested in Pacific white shrimp *Litopenaeus vannamei* compared to other ingredients such as fishmeal, krill meal, soybean meal and polychaete meal (Jormasie 2009). The digestibility coefficients of peaseed meal were as high as 88% and 94% for energy and protein respectively compared to the slightly lower digestibility of 82% and 85% for fishmeal.

The present study also shows that feed intake of fish fed the MPS meal (which consists of mainly soybean) was at 6.4% lower than that of fishmeal (Figure2). This indicates that higher inclusion levels of soybean lead to a decrease in feed intake. This result agrees with the findings of Goda et al. (2007) who found that feed intake of Nile tilapia fingerlings *O. niloticus* fed 100% of soybean was lower in fish fed a fishmeal control.

The present investigation found that the growth performance of tilapia fed MPS (containing soybean supplemented with lysine, methionine, and threonine) was still lower than those fed a fishmeal diet (Figure 1). This result supports the findings of the study of Goda et al. (2007) who found that the growth rate of Nile tilapia fingerlings *O. niloticus* fed 100% of soybean supplemented with L-lysine and DL-methionine was lower than Nile tilapia fed fishmeal diet. In another study, 100% of fishmeal protein in diets for Nile tilapia fingerlings could be totally replaced with soybean protein meal supplemented with essential amino acids (lysine, methionine, threonine) without negative effects on the growth. As weight gain of Nile tilapia fed fishmeal only diet was similar to a weight gain of Nile tilapia fed soybean supplemented with the essential amino acids (Furuya et al. 2004).

The other observation of the present study was the comparison between feed intake and subsequent growth rate in tilapia when fed the polymeal and the MPS feeds. Performance of fish fed both diets were still inferior to the fishmeal feed. However, contrary to what might be expected, polymeal (based on a blend of soybean and polychaete meal) was not outperforming the MPS feed (based on soybean only). Feed intake of tilapia fed the polymeal diet was with 6.60% per day lower than in fish fed the fishmeal diet (Figure 2). The lower feed intake subsequently resulted in lower growth. This result does not agree with findings of Davies & Gouveia (2010) who investigated the substitution of fishmeal at increasing levels (0%, 25%, 75%, 100%) of pure polychaete worm meal derived from *Nereis virens* on the growth of shrimp *L. vannamei*. The investigation found that feed intake and growth in shrimps fed a diet based on 100% polychaete meal was comparable to the performance when fed the fishmeal only diet. Polychaete worms might serve as a feed attractant as several studies have shown. Polychaete worms were more palatable than formulated diet in feeding experiment for Chinese shrimp *Fenneropenaeus chinensis*, as feed intake of Chinese shrimp fed polychaete worms was higher when compared to that of a regular formulated diet (Guoqiang et al. 2005). Rijnsdorp & Vingerhoed (2001) reported that additional polychaetes had led to an improvement of the diet for the two flatfish species plaice *Pleuronectes platessa* L and sole *Solea solea* (L) in heavily trawled areas, when compared to the beginning of the 20^{th} century. To the contrary, the present study shows that feed intake and growth performance of tilapia fed the polymeal diet was not improved compared to the one fed of the Marine Protein Substitute meal (MPS). This result might suggest that the poly meal diet which - according to the manufacturer – contains a mixture of soybean, polychaete worms, and seaweed might contain only traces of polychaete meal however not enough to enhance the feed intake similar to fishmeal fed tilapia.

Whole body composition of tilapia fed the different diets confirmed former results of Lupatsch (2008) and Lupatsch et al. (2003) who suggested that protein and ash content in fish is relatively constant. In contrast, energy and moisture content are changing according to fish size. In the present study (apart from fish fed the peaseed meal) tilapia fed the different diets had a protein content ranging from 161 mg per g live weight (Table5). This result agrees with the finding of Lupatsch (2008) and Schulz et al. (2007) who found that the level of protein content in tilapia was 160 mg per g body mass.

In addition, the present study showed that protein content of tilapia fed peaseed meal Diet 3 was the lowest (143mg/g) in comparison to tilapia fed the other diets. This result supports the findings of El-Saidy & Saad (2008) who reported that the level of the protein content of Nile tilapia *O. niloticus* was decreased from (17.0% to 15.2%) with increasing replacement of fishmeal with cow peaseed meal in their diet. The reduction of whole body protein in the present study might be due to the low consumption of the peaseed meal diet, as the lower feed intake would reduce the availability of protein for growth.

In the present study, it can be also seen that the level of the dry matter content of tilapia fed peaseed meal was the lowest 268 mg/g compared to the level of dry matter of tilapia fed the other diets (Table 5). The lower level of dry matter content was associated with lower levels of lipid and energy content. The lower dry matter and lipid and energy were related to a low feed intake of peaseed meal.

Protein gained was found to be lowest in tilapia fed peaseed protein (0.03g/tilapia/day), in comparison to those fed fishmeal (0.13 g/tilapia/day) (Table 7). Protein gained was positively related to crude protein intake. This result was supported by the finding of Lupatsch (2008) who reported that the relationship between protein intake and protein gained was correlated in tilapia, as protein gain increased linearly with increasing protein intake.

However, protein retention efficiency was found to be highest at 38.5% in tilapia fed peaseed meal, whereas tilapia fed the fishmeal diet had the lowest value of protein retention efficiency (29.8%). This result agrees with the findings of Gouveia & Davies (2000) who reported that the high inclusion level of peaseed meal had led to an enhanced protein assimilation which was the main reason for an increased protein retention efficiency. In the present study, the high protein retention efficiency was probably related to the limiting supply of protein. This might be because all the protein consumed by the fish was used exclusively for protein synthesis and not as an energy source. In agreement to this Lupatsch et al. (2003) found a negative relationship between protein intake and protein retention efficiency in seabream but only when dietary protein was supplied above requirement.

Energy gained was found to be lowest in tilapia fed Diet3 (1.23 kJ/tilapia /day) compared to those fed Diet1 (5.82 kJ/tilapia/day) (Table 6). Energy gained was as well positively correlated to gross energy intake. This result agrees with the finding of Lupatsch (2008) who showed that the amount of energy gained in tilapia was positively correlated with the amount of energy consumed until the fish refused to feed more. But, contrary to the protein efficiency, the energy retention efficiency of tilapia fed the four different diets were not significantly different.

The survival rate throughout the experiment was varied but not significantly different. Fish fed Diet 2 showed the highest survival rate (97.8% ± 3.85), and fish fed Diet1 the lowest (71.1% ± 23.41). These variations were not related to dietary treatment, but were due to the aggressive encounters that might be caused by the low stocking density. According to Kjartansson et al. (1988), aggression was reduced in Atlantic salmon *Salmo salar* when kept at higher stocking densities. The stocking density and social interaction had more pronounced effects on the stress response of Nile tilapia *O. niloticus*, a density between 5 and 10 fish in the tank was the source of stress for Nile tilapia fingerlings when compared to other stocking densities (Barcellos et al. 1999).

CONCLUSIONS

By investigating the peaseed meal, polymeal and Marine Protein Substituted (MPS) as a replacement for fishmeal in the diet of Genetically Male Tilapia (GMT), it has been found that the highest feed intake and growth performance were obtained in tilapia fed fishmeal. In contrast, the lowest feed intake and growth performance were achieved in tilapia fed the peaseed meal. Tilapias fed polymeal and MPS showed the same feed intake and growth performance, but was lower than achieved when fed with fishmeal.

Tilapia fed the peaseed meal had the best FCR of 0.86 and the highest protein retention efficiency of 38.5%. This suggests that peaseed meal as a protein source was used effectively by tilapia, however, feed consumption was low and was thus depressing the growth. Considering the economics it can be concluded that fishmeal could be replaced by polymeal or MPS meal without adverse effects, however, the low palatability of peaseed meal would require an additional attractant in the feed.

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APPENDICES

Table 1: Composition of Feed Ingredients Used (Per kg on as Fed Basis)

	Dry Matter (g)	Crude Protein (g)	Lipid (g)	Ash (g)	Phosphorus (g)	Gross Energy (MJ)
Fishmeal[1]	933	622	100	168	26.8	18.34
Polymeal[2]	920	512	23	57	7	18.80
Peaseed meal[3]	930	751	65	51	6	21.68
MPS[4]	920	512	17	46	6	18.82
Rapeseed oil	990	0	980	0	0	38.39
Wheat starch	900	0	0	15	0	15.05

[1] Fishmeal - source Argentina

[2] Polymeal - source Dragon Feed Ltd, UK

[3] Peaseed meal - source Roquette, France

[4] Marine Protein Substitute - source Dragon Feed Ltd, UK

Table 2: Formulation of the Experimental Diets (Per kg as Fed)

	Diet1 Fishmeal	Diet2 Polymeal	Diet3 Peaseed meal	Diet4 MPS
	(g)	(g)	(g)	(g)
Fishmeal	650			
Polymeal		780		
Peaseed meal			530	
MPS meal				780
Starch	265	40	310	40
Vitamin and mineral mix[1]	10	10	10	10
CaSO4	20	20	20	20
Alginate[2]	20	27	27	27
DCP[3]	0	50	50	50
Rapeseed oil	35	80	60	80

[1]Vitamin and Mineral premix for shrimps, source DSM - nutritional products

[2] Binders

[3] DCP- Dicalcium phosphate - source of phosphorus

Table 3: Analyzed Composition of the Feeds (Per kg as Fed)

	Dry Matter	Crude Protein	Lipid	Ash	Gross Energy
	(g)	(g)	(g)	(g)	(MJ)
Diet1	975	425	96	166.9	17.71
Diet2	948	422	99	112.2	18.38
Diet3	957	416	102	101.2	19.00
Diet4	950	415	92	103.6	17.86

Table 4: Performance of Tilapia Fed the Four Experimental Diets (Mean ± SD of Three Replicate Treatments) after 32 Days of Growth

	Initial Weight	Final Weight	Weight Gain	Feed Consumed	FCR	Survival
	(g)	(g)	g/fish/day	g/fish/day		(%)
Diet1	5.74	31.37	0.80[a]	1.01[a]	1.25 [a]	71.1[a]
	±0.08	±1.54	±0.05	±0.10	±0.05	±23.4
Diet 2	5.67	26.67	0.66 [b]	0.81[b]	1.24[a]	97.8[a]
	±0.22	±2.02	±0.06	±0.08	±0.02	±3.8
Diet3	5.73	13.45	0.24[c]	0.21[c]	0.86[b]	84.4[a]
	±0.30	±0.53	±0.01	±0.02	±0.07	±15.4
Diet4	5.77	26.72	0.65[b]	0.80 [b]	1.22[a]	91.1[a]
	±0.30	±1.66	±0.05	±0.06	±0.03	±15.4

[a,b,c] values in each column with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey Test)

Table 5: Composition of Tilapia per G Live Weight Fed the Experimental Diets (mean ± SD, n=3)

	Dry matter	Protein	Lipid	Ash	Energy
	mg	mg	mg	mg	kJ
Initial	301.5	150.7	117.2	35.5	7.37
Diet1	296 ± 11.2[a]	158 ± 7.56[a]	105.1 ± 6.0[a]	38.4 ± 0.72[b]	7.29 ± 0.34[a]
Diet2	285 ± 2.4[a,b]	161 ± 3.21[a]	89.2 ± 5.4[a,b]	37 ± 0.87[b]	6.88 ± 0.18[a,b]
Diet3	268 ± 8.05[b]	143 ± 1.24[b]	77.3 ± 7.0[b]	47 ± 2.72[a]	6.08 ± 0.40[b]
Diet4	279 ± 10.7[a,b]	160 ± 1.40[a]	84.7 ± 11.0[b]	36 ± 1.13[b]	6.70 ± 0.32[a,b]

[a,b,c] values in each column with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey Test).

Table 6: Efficiency of Energy Retention (mean ± SD) in Tilapia fed Four Experimental Diets

	Gross Energy Intake (kJ/tilapia/day)	Energy Gained (kJ/tilapia/day)	Energy Retention Efficiency (%)
Diet1	17.77 ± 1.78[a]	5.82 ± 0.09[a]	32.94 ± 2.97[a]
Diet2	14.89 ± 1.57[a,b]	4.43 ± 0.47[b]	29.77 ± 1.45[a]
Diet3	3.92 ± 0.29[c]	1.23 ± 0.17[c]	31.31 ± 2.15[a]
Diet4	14.17 ± 1.09[b]	4.27 ± 0.52[b]	30.05 ± 1.55[a]

[a,b,c] values in each column with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey Test)

Table 7: Efficiency of Protein Retention (mean ± SD) in Tilapia Fed Four Experimental Diets

	Crude Protein Intake (g/tilapia/day)	Protein Gained (g/tilapia/day)	Protein Retention Efficiency (%)
Diet1	0.426 ± 0.04[a]	0.13 ± 0.00[a]	29.8 ± 3.02[b]
Diet2	0.342 ± 0.04[b]	0.11 ± 0.01[b]	31.5 ± 0.66[b]
Diet3	0.086 ± 0.01[c]	0.03 ± 0.00[c]	38.5 ± 3.19[a]
Diet4	0.330 ± 0.03[b]	0.11 ± 0.01[b]	32.2 ± 0.52[b]

[a,b,c] values in each column with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey Test)

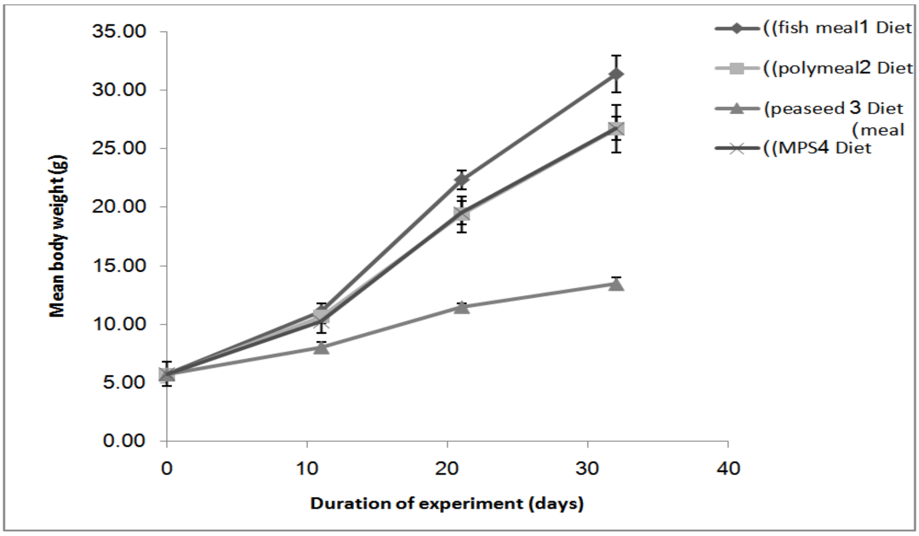

Figure 1: Growth of Tilapia Fed the four Experimental Diets Over a Period of 32 Days (mean values ± SD, n = 3)

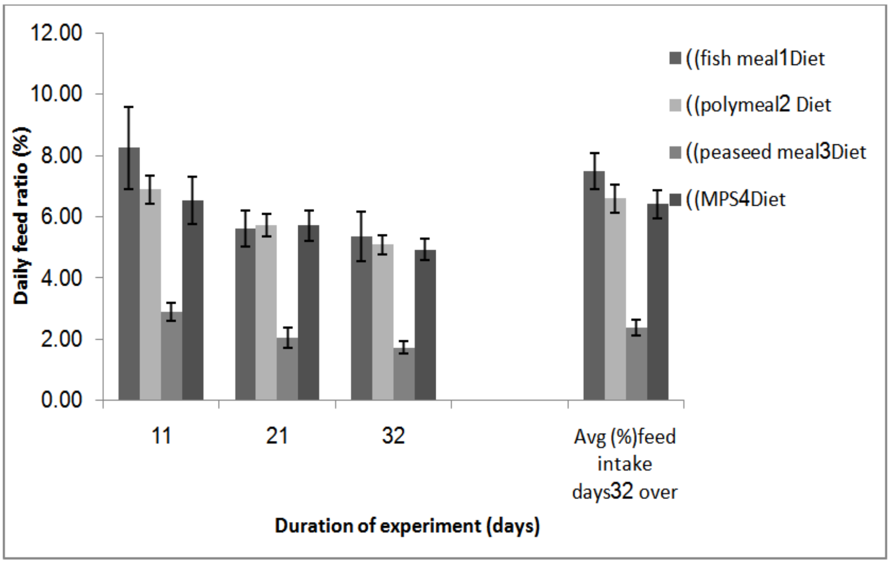

Figure 2: Daily Feed Ration (%) of Tilapia Fed the Four Experimental Diets Calculated for Each Weighing Period and Mean Overall % Feed Intake for the Duration of the Whole Trial (mean, ± SD)