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EFFECT OF REPLACING FISH MEAL WITH MEAT AND BONE MEAL IN NILE TILAPIA *(O. niloticus)* DIETS. 1-NUTRITIONAL EVALUATION AND SAFETY ASSESSMENT STUDY

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ABSTRACT

A growth trial was conducted firstly, to evaluate the effects of using meat and bone meal (MBM) instead of fish meal at rate of 0, 25, 50, 75 and 100% in diets on the growth performance and feed efficiency of Nile tilapia (*Tilapia niloticous*). Secondly, to assess the safety of inclusion MBM as animal protein source in fish feeds. Five iso-nitrogenous (32% crude protein) and iso-energetic (3500 Kcal estimated digestible energy) diets replacing 25, 50, 75 and 100% of fish meal by MBM were formulated. Each diet was randomly allocated to duplicate groups of fish in fiberglass tanks and each tank was stocked with 25 fingerlings (initial average body weight 1.80 ± 0.15g). Fish were hand fed the experimental diets four times per day for 60 days. For microbial safety assessment of examined fish, three samples from each group were collected at the end of the experiment in sterile bags to measure total bacterial count, total faecal coliform count, bacillus cereus, salmonella and staphylococca. There were no significant differences in terms of final body weight, average weight gain percentage and specific growth rate among fish fed the control diet and those fed diets contained meat and bone meal up to 50% replacement. There were also no significant differences in terms of feed intake, feed conversion ratio and protein efficiency ratio between fish fed the control diet and those contained 25% replacement of fish meal with meat and bone meal. The lowest performance had been recorded for the group of fish fed diet containing 100% meat and bone meal. Total Coliform Count, Faecal Coliform count, Bacillus cereus and Salmonella were not detected in all examined samples. The obtained results of Staphylococcal count showed no significant difference between all groups. The economical efficiency study demonstrated that replacing 25% of fish meal with meat and bone meal had the best net revenue 60.92 L.E. followed by the control 60.88L.E. and 50% replacement of fish meal 58.98 L.E.

Although the present results showed that MBM could safely replace up to 50% of fish meal content in Nile tilapia diets without any adverse effect on Nile tilapia performance and its safety use. Yet, the 25% replacement was the most economical.
Keywords: fish meal, meat and bone meal, nutrition evaluation, safety assessment

INTRODUCTION

Aquaculture is the fastest growing animal protein production sector in the world since 1970, with an annual growth rate of 6.9%. Egypt is ranked to be from the first 27th aquaculture producers all over the world with total annual production 1.4 million tons.

Feed represents 40-70% of operating cost. Fish meal is the most important and most expensive protein for commercial aquaculture feed. It provides fish with essential amino acids, fatty acids and trace mineral (USB, 2008). Also, fish meal is highly palatable for fish (Li *et al.* 2006) and promotes optimum growth performance (Gomes *et al.* 1995). Unfortunately, fish meal supply is limited due to increasing demand and decreasing marine fishery resources.Therefore, alternative protein sources, including plant and animal proteins, have been studied by many fish nutritionists (Tacon and Jackson 1985). However, plant proteins usage is limited as they are difficent in some essential amino acids, as well as the presence of anti-nutriental factors and/ or poor palatability. Therefore, It was suggested that at least one animal protein source must be found in fish diets to cover fish's amino acid requirements (Gomes *et al.* 1995).

Meat and bone meal (MBM) is a potential animal protein source to be used in fish feeds because of its' high protein content (47.3 – 54.3% dry base), excellent amino acid profile and compatible price comparing to fish meal (Ferouz, *et al.* 2012). Previous studies have shown that MBM could successfully substitute fish meal up to 30% in fish feeds (Pongmaneerat and Watanabe 1991; Robaina *et al.* 1997; Bureau *et al.* 2000; Kureshy *et al.* 2000). Higher replacement levels have been reported in gilthead sea bream *Sparus aurata* (Alexis 1997), Mozambique tilapia *Oreochromis mossambicus*, (Davies *et al.* 1989) and rainbow trout *Oncorhynchus mykiss* (Watanabe and Pongmaneerat 1991).

However, since 2001 there has been a total ban on the use of mammalian meat and bone meal in animal feed due to the presence of prion protein which is expected to be the principle cause of Transmissible Spongiform Encephalopathies (TSE) in animals (FAO, 2004). As a result, many studies have been conducted using different animal species, including fish, as recipients of a TSE agent to answer public concern about safety of food possibly contaminated with TSE agents (NRA, 2009).

Liao *et al.* (2005) reported that the passage of TSE agents between animals of different species is usually impaired by the species barrier, Dalla Valle *et al.* (2008) and Chiesa and Harris (2009) reported that, prion could persist in intestine and caecal submucosa and didn't cross the intestinal barrier.

In 2012 FAO has proposed the lifting of the ban for using meat and bone meal only in fish feed.

The aim of the present study is therefore to evaluate the potential of using MBM as a substitute for fish meal keeping in mind the availability of fish meal in markets and to assess the microbial safety of using meat and bone meal in tilapia diets.

MATERIALS AND METHODS

Experimental system and fish:

Nile tilapia fingerling *(Oreochromis niloticus)*, mono sex were brought to the Fish Experimental Unit at Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt, from a fresh water commercial farm in Damietta governorate.

The fish were reared in a closed-recalculating water system. The study was done in 10 fiberglass tanks from this system with capacity of 60 L water each. Water flow out of each aquaria at 2L/min into a submerged bio-filter after passing through a mesh net to remove solid impurities. Water was then collected in a common reservoir from which the filter water is pumped up to the rearing units. The water used in the system was stored-tap water, which was aerated using a blower aerator-type. Five percent of the total water volume was renewed daily. A thermo-controlled electric heater was used to adjust water temperature about 24±1ºC. All the experimental treatments were conducted under an artificial photo period equal to natural light/darkness period (12h light:12h darkness).

Diet formulation

Five experimental diets were formulated to contain ~32% crude protein and ~3500 Kcal estimated digestible energy according to NRC, 1993 (Table 1). The control diet was formulated to contain fish meal and soybean meal as the primary protein sources (D1).The other four experimental diets were formulated to replace 25%, 50%, 75% and 100% of fish meal with meat and bone meal (D2, D3, D4 and D5,,respectively).

The proximate analysis, amino acids and minerals of the experimental diets were analyzed according to (AOAC, 2005).While the digestible energy were calculated according to Wang *et al.,* (1985) (Table 1). Calcium and phosphorus were adjusted using monocalcium phosphate. Vitamins and trace minerals were added.

Table(1): Composition of experimental diets (on fed basis).

Items	D1	D 2	D3	D 4	D 5
Fish meal (FM) 60%	12.00	9.00	6.00	3.00	……..
Meat and bone meal	…….	3.00	6.00	9.00	12.00
Soybean meal 48%	43.00	44.00	45.00	46.00	47.00
Ground yellow, corn.	18.40	18.40	18.40	18.40	18.40
Wheat bran	8.50	8.50	8.50	8.50	8.50
Corn starch	9.00	8.50	8.10	7.70	7.30
Grounded mung bean seeds	5.00	5.00	5.00	5.00	5.00
Monocalcium phosphate	2.50	2.00	1.40	0.80	0.20
Vit. & min. mix*	1.60	1.60	1.60	1.60	1.60
Total	100	100	100	100	100

*** Vitamin and mineral premix at 2.5% of the diet supplies the following per kg of the diet: vit. A 75000 IU, Vit. D 9000 IU, vit. E 150mg, vit. K 30mg, vit. B_1 26.7mg, vit. B_2 30mg, vit. B_6 24.7mg, vit. B_{12} 75mg, niacin 225mg, pantothenic acid 69mg, folic acid 7.5mg, vit. C 150mg, choline chloride 500mg, Mn 204mg, Fe 93mg, Zn 210mg, Cu 11.25mg, I1.02mg,**

Experimental procedure:

Two hundred and fifty Nile tilapia fingerlings *(Oreochromis niloticus)* mono sex of mean initial body weight (1.80 ± 0.15g) were randomly distributed on 10 open system 60 liter tanks, where each tank contained 25 fingerlings. Each two tanks (duplicate) represented an experimental treatment. The first 15 days of the experiment were considered as habituation period and thereafter the growth trials were carried out for 60 days. Diets were randomly assigned to the experimental units. Fish were hand fed the experimental diets at three% rate of body weight for six days weekly, four times per day **(**Jauncey and Ross, 1982 and Coche**,** 1982**)**. Fish were weighed every two weeks.

Growth parameters and feed efficiency:

Growth and nutrient utilization parameters were recorded and analyzed in terms of initial body weight (IBW), final body weight (FBW), weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). Mortality percentage was calculated at the end of the trial.

Microbial safety assessment of examined fish:

Three samples from each group were collected at the end of the experiment in sterile bags and were put in ice box and sent to the laboratory. Subsamples were taken under complete aseptic conditions and were prepared according to (NMKL 2001a). Ten-fold dilutions were prepared using sterile saline solution. Specific media was used for enumeration of all microbial parameters using pour plate technique as; Plate count agar was used for total bacterial count (NMKL 1999a)**,** Violet Red Bile agar (VRB) for Total (NMKL 2001b) faecal coliform count (NMKL 1996), Baird Parker agar for Staphylococcal count (NMKL 1999b)**,** Bacillus cereus selective agar for Bacillus cereus (NMKL 1997), Brillient Green for Salmonella spp. (NMKL 1999c) and Rose Bengal Chloramphenicol agar for Total Fungal Count (NMKL 1995).

Economical evaluation

The use of meat and bone meal in Tilapia diets has been economically evaluated to measure the impact of such practice on the performance efficiency.

The following equations were used to calculate net revenue, economical efficiency and relative economical efficiency of various experimental diets.

Net revenue = Total income (L.E.) – Total feed cost (L.E.)

Were Total income(L.E.)=Total final fish weight Kg*price of one Kg of fish (L.E.)

Total feed cost = Total amount of feed consumed Kg * price of one Kg diet (L.E.).

Economic efficiency = Net revenue (L.E.) / Total feed cost

Relative economical efficiency = calculated as a percentage from the economical efficiency of the control diet.

Statistical analysis:

The obtained data were subjected to a two way analysis of variance using the linear model (GLM) of SAS (SAS Institute, 1991). Means were compared using Duncan's new multiple range test ($P<0.05$) (Duncan, 1955).

RESULTS AND DISSCUSSION

Chemical composition of the experimental diets:

The chemical composition (Table 2) of the experimental diets showed limited variations among these diets; also there was limited variation in methionine, ceystine and lysine contents.

Table(2): Chemical composition of experimental diets (on fed basis).

Items	D1	D2	D3	D4	D5
Dry matter (%)	92.40	92.10	92.60	92.00	92.00
Crude protein (%)	32.50	31.95	32.35	32.00	32.10
Ether extract (%)	1.58	2.13	2.28	2.39	2.84
Crude fiber (%)	3.08	3.05	3.09	3.22	3.31
Ash (%)	8.02	7.96	8.08	8.31	8.62
NFE (%)[1]	47.22	47.01	46.80	46.08	45.13
Digestible energy (Kcal/kg)[2]	3494	3501	3499	3506	3511
Total (phos.) (%)	1.36	1.37	1.37	1.37	1.37
calcium (%)	1.35	1.33	1.35	1.33	1.35
Lysine	2.09	1.95	1.87	1.78	1.69
Methionine	0.60	0.57	0.54	0.53	0.55
Cystine	0.69	0.50	0.48	0.50	0.50
Protein energy ratio (mg/kcal)[3]	93.02	91.26	92.45	91.27	91.43

[1] **NFE = 100 – (% moisture + % protein + %EE + %ash + % Fibre).**

[2] **Digestible energy was calculated using the values 4.5, 4 and 9 kcal /g for protein, carbohydrate and lipid, respectively according to Wang *et al.,* (1985)**

[3] **Protein energy ratio (P/E ratio) = crude protein x 10000 / digestible energy, according** to **Hepher *et al.,*(1983)**.

Growth performance:

The present data (Table 3), showed that there was no significant difference (P>0.5) in the average initial body weight among fish used in the trial in all groups.

There were also no significant (P>0.05) differences in term of final body weight, average weight gain percentage and specific growth rate between fish fed the control diet and those fed diets contained meat and bone meal up to 50% replacement.

Table(3): Effect of replacing dietary fish meal with meat and bone meal on tilapia growth performance and feed efficiency [1].

Experimental treatments	Replacement levels	IBW[2]	FBW[3]	FI[4]	WG%[5]	FCR[6]	PER[7]	SGR[8]
Diet 1	-----	1.95 ±0.10	9.50[a] ±0.37	13.62[a] ± 0.24	387.18[a] ±10.35	1.80[c] ±0.03	1.71[a] ±0.04	2.83[a] ±0.06
Diet 2	25%	1.90 ±0.11	9.19[ab] ±0.01	13.27[ab] ±0.16	383.36[a] ±8.56	1.82[bc] ±0.11	1.72[a] ±0.04	2.80[a] ±0.03
Diet 3	50%	1.80 ±0.10	8.51[ab] ±0.40	12.61[bc] ±0.25	372.77[a] ±8.87	1.88[b] ±0.09	1.64[b] ±0.05	2.76[a] ±0.06
Diet 4	75%	1.67 ±0.10	7.34[bc] ±0.36	11.28[c] ±0.23	339.52[b] ± 4.1	1.99[a] ±0.04	1.57[c] ±0.03	2.64[b] ±0.05
Diet 5	100%	1.65 ±0.10	6.43[c] ±0.4	9.66[d] ±0.25	289.69[c] ±12	2.02[a] ±0.07	1.54[c] ±0.06	2.42[c] ±0.06

[1] values are the mean of duplicate groups of fish. Mean values in columns with different superscripts are significantly different (P<0.05).

[2] IBW Initial body weight (g). [3] FBW Final body weight (g). [4] FI Feed intake (g).

[5] WG weight gain (%). [6] FCR Feed conversion ratio (g/g). [7] PER Protein efficiency ratio (%).

[8] SGR Specific growth rate (%).

The results showed that there were no significant differences (P>0.05) in term of feed intake, feed conversion ratio and protein efficiency ratio between fish fed the control diet and that containing 25% replacement of fish meal with meat and bone meal.

These results were in agreement with the finding of (Robaina *et al.* (1997); Wu *et al.* (1999); Yang *et al.* (2004) and Ai, *et al.* (2006)) who concluded that, MBM could replace fish meal up to 50% without any negative effects on growth.

The least performance had been recorded for the fish fed diet contained 100% replacement of fish meal with meat and bone meal when compared with fish fed the control diet and diets contained either 25% replacement of fish meal with meat and bone meal or 50% replacement. These differences were statistically significant (P<0 .05)

This trend agrees well with the finding Yang *et al* (2004) and Ai, *et al.* (2006) who reported that a depression in growth performance occurred when meat and bone replaced fish meal by more than 50%. Watanabe and Pongmaneerat (1991) attributed this depression in growth performance to the poor digestibility and imbalance of essential amino acids of meat and bone meal. In a balanced amino acid diets trial Yamamoto *et al.* (2002) found that still a depression in growth performance when the replacement percentage of fish meal by meat and bone meal exceeded 50%. They related this depression to the high ash content of meat and bone meal which may produce a faster gut transit rate, thus providing an increased feed intake with poor reflection on growth and thus, poor feed efficiency.

On the contrary, Davies *et al.* (1989); Watanabe and Pongmaneerat (1991) and Millamena (2002) successfully replaced 750, 800 and 900 g/kg) of fish meal protein with MBM combined with other protein sources in diets for Mozambique tilapia, grouper *Epinephelus coioide* and rainbow trout, respectively. These higher acceptable replacement levels of meat and bone might be due to differences in quality of MBM and animal protein blends tested.

Microbial safety assessment:

The present data Table (4) illustrate that there was no significant difference in Total Bacterial Count in all treated groups compared to the control group.

Table (4) Microbial quality parameters in fish fed different levels of MBM:

Test / Treatment	TPC Cfu/g	TCC Cfu/g	FCC Cfu/g	Staph Cfu/g	B. cereus Cfu/g	Salmoela Cfu/g	TFC Cfu/g
Control	29x10	-	-	2x10	-	-	-
25%	47x10	-	-	7x10	-	-	-
50%	17x10	-	-	8x10	--	-	-
75%	13x10	-	-	4x10	-	-	-
100%	56x10	-	-	15x10	-	-	3x10

TPC= Total Plate count TCC= Total Coliform Count FCC= Faecal Coliform Count
Staph=Staphylpcoccal count B. cereus= *Bacillus cereus* count
TFC= Total Fungal County
CFU= Colony Forming Unit

Numerical increase in the number of colony forming unit was recorded in the group given 100% MBM as protein source. This observation has no significant negative effect on the judgment on the end product's microbial quality as the TFC still far below the permissible limits 3log Total Coliform Count, Faecal Coliform count, Bacillus cereus and Salmonella were not detected in all examined samples. The obtained result of Staphylococcal count showed no significant difference among all groups compared to the control group but cfu/g number increased in group fed the 100% MBM as protein source by one log.

All treated groups showed negative result of Total Fungal Count while very few colonies was found in group fed on 100% MBM as protein source.

The present data revealed that, using of MBM as protein source in fish diet showed no microbial health hazard as the obtained microbial quality was considered to be of good status and the absence of pathogenic microorganisms and the low quantity of indicator parameters (TCC, FCC, *Staphylococi* and *Bacillus cereus*) indicates the efficient processing of this protein source. The data presented by (FAO, 2004) (Table 5) supported this conclusion.

Table (5): Efficacy of rendering system in the destruction of pathogenic bacteria (FAO,2004).

Pathogen	Pre rendering	Post rendering
Clostridium perfringens	71.4 %	0 %
Listeria species	76.2 %	0 %
L. monocytogenes	8.3 %	0 %
Campylobacter species	29.8 %	0 %
C. jejuni	20.0 %	0 %
Salmonella species	84.5 %	0 %

Data obtained in the above table show that, heat treatment of meat and bone meal can destroy all micro biological hazards even those which are resistible to unfavorable conditions; like clostridium perfringins bacteria which is gram positive (has thick cell wall) spore forming bacteria giving an idea about the safety usage of it as protein source in fish feeding.This results agreed with those obtained of Miles and Jacob (1998) and Yu (2004).

Economical evaluation:

Table (6) illustrated that the most economical diet was D2 containing 3% meat and bone meal (replacing 25% fish meal), which gave net revenue 60.92 L.E. followed by D1 60.88 L.E. than D3 58.98L.E. the worst net revenue presented by D5 (replacing 100% fish meal) 46.34 L.E.

Table (6): Economical efficiency of Nile tilapia *(tilapia niloticous)* fed the experimental diets.

Items	D1	D2	D3	D4	D5
Total amount of feed consumed (Kg).	13.62	13.27	12.61	11.28	9.66
Price of one Kg diet (L.E.)	3.90	3.72	3.55	3.37	3.19
Total feed cost (L.E.)*	53.12	49.36	44.14	38.01	30.82
Total fish weight (Kg)	9.50	9.19	8.51	7.34	6.43
Price of one kg fish (L.E.)**	12.0	12.0	12.0	12.0	12.0
Total income (L.E.)	114.0	110.28	102.12	88.80	77.16
Net revenue (L.E.)	60.88	60.92	58.98	50.79	46.34
Economical efficiency	115	124	134	134	150
Relative economical efficiency (%)	100	108	117	117	130

*** Economic evaluation was calculated depending on the prevailing prices being : price of D1, D2, D3, D4 and D5 was 3900, 3720, 3550, 3370 and 3190 L.E. respectively,**
**** However Kg of tilapia fish was 12 (LE).**

The economical efficiency could be used to compare the differences among the experimental treatments. The priority of the diets goes to the more economical ones.

The results showed that diets containing meat and bone meal (D2, D3, D4 and D5) scored the least feed cost (L.E.) values, when compared to the control group. The least value (30.82) was for 100% meat and bone meal (D5) while, the highest value (53.12 L.E.) was for 100% fish meal (D1 control). The best relative economical efficiency (130%) was for the group fed 12% meat and bone meal (D5) followed by 6% and 9% meat and bone meal (D3 and D4) (117% for each).It could be concluded that replacing 25% fish meal with meat and bone meal in Nile tilapia dites can be used to get better net revenue (LE).

CONCLUSION

Although the present study indicated that meat and bone meal could be used as an animal protein source to replace fish meal up to 50%, without adverse effects. Yet 25% replacement was the most economical. More work is needed however to explore the possibility of reducing the ash content of MBM and improving the digestibility of meat and bone meal by improving processing methods.

REFERENCES

Ai, Q.; Mai, K., Tan, B., Xu, W., Duan, Q., Ma, H. and Zhang, L. (2006). Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea*. Aquaculture, 260, 255-263.

Alexis, M.N. (1997) Fish meal and fish oil replacers in Mediterranean marine fish diets. In: Feeding Tomorrow's Fish. Proceedings of the workshop of the CIHEAM network on technology of aquaculture in the Mediterranean (Tacon, A. & Barsureo, B. eds), CIHEAM, Zaragoza, Spain, 1989, pp. 183–204.

A.O.A.C., (2005). "Official methods of analysis of the Association of official Analytical Chemists", 18th. Ed. Vol. 1, published by the A.O.A.C., Benjamin Francklin station, Washington, DC.

Bureau, D.P.; Harris, A.M., Beven, D.J., Simmons, L.A., Azevedo, P.A. and Cho, C.Y. (2000). Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (Oncorhynchus mykiss) diets. Aquaculture, 181, 281–291.

Chiesa R. and Harris D.A (2009) Fishing for Prion Protein Function. PLOS Biology 7(3): e75

Coche, A.G. (1982). Cage culture of tilapias. In: the Biology and Culture of Tilapia, eds. R.S.V. Pullin and R.H. Lowe-McConnel. Manila, Philippines: ICLARM. Cited by Lovell, T. (1989).

Dalla Valle A.Z., Iriti M., Faoro F., Berti C. and Ciappellano S. (2008). *In vivo* prion protein intestinal uptake in fish. APMIS (116): 173-180

Davies, S.J.; Williamson, J., Robinson, M. and Bateson, R.I. (1989) Practical inclusion levels of common animal by-products in complete diets for tilapia Oreochromis mossambicus, Peters. In: The Current Status of Fish Nutrition in Aquaculture Proceedings of the Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan, 28 August–1 September, Japan, 1989 (Takeda, M. & Watanabe, T. eds), pp. 325–332.

Duncan,D.B., (1955).Multiple range and Multiple F test. Biometrics,11:1 – 42.

FAO, (Food and Agriculture Organization of the United Nations) (2004). Protein Sources for Animal Feed Industry, Expert Consultation and Workshop, Bangkok, 29 April – 3 May 2002 FAO Animal production and health proceedings.

Ferouz, Y. A.; Kurt, A.R. and Kasiviswanathan, M. (2012). Alternative protein sources for aquaculture feeds. J. Aquaculture feed Science and Nutrition, 4(1): 1 – 26.

Gomes, E.F.; Rema, P., and Kaushik, S.J. (1995). Replacement of fish meal by plant proteins in the diet of rainbow trout *(Oncorhynchus mykiss)*: Digestibility and growth performance. Aquaculture, 130: 177 – 186.

Hepher, B.; Liao, I.C. Cheng, S.H. and Haseih, C. S. (1983). Food utilization by red tilapia. Effect of diet compostion, feeding level and temperature on utilization efficiency for maintenance and growth. Aquaculture, 32: 255 – 272.

Jauncey, K. and Ross, B.R. (1982). “A guide to tilapia feeds and feeding”. Institute of Aquaculture, University of Striling. FKA, Scotland, V.K.L., 9: 68.

Kureshy, N.; Davis, D.A. and Arnold, C.R. (2000) Partial replacement of fish meal with meat and bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. N. Am. J. Aquacult., 62, 266– 272.

Li, M.H.; Peterson, B.C., Janes, C.L. and Robinson, E.H. (2006). Comparison of diets containing various fish meal levels on growth performance, body composition and insulin-like growth factor – I of juvenile channel cat fish *Ictalurus puctatus* of different strains. Aquaculture, 253: 628 – 635.

Liao M.; Zhang, Z., Yang G., Sun X., Zou G., Wei Q., and Wang D. (2005). Cloning and characterization of prion protein coding genes of Japanese seabass (*Lateolabrax 374aponicas*) and Japanese flounder (*Paralichthys olivaceus*). Aquaculture 249: 47– 53

Millamena, O.M., (2002) Replacement of fish meal by animal by-product meals in practical diet for grow-out culture of grouper Epinephelus coioides. Aquaculture, 204, 75–84.

Miles, R.D., and Jacob, J.P. (1998). Using meat and bone meal imn poultry diets. Edis.ifas.Ufl.Edu/pdffiles/PS/PS02400.PDF.

Nordic Committee on Food Analysis (NMKL) 1995, Mould and yeasts. Determination in foods. 98 1995, 3rd Ed.

Nordic Committee on Food Analysis (NMKL) 1996, Coliform bacteria, thermotolerant. Enumeration in foods. 125 1996, 3rd Ed.

Nordic Committee on Food Analysis (NMKL) 1997, Bacillus cereus. Determination in foods. 67 1997, 4th Ed.

Nordic Committee on Food Analysis (NMKL) 1999a, Aerobic microorganisms. Determination in foods. 86 1999a, 3rd Ed.

Nordic Committee on Food Analysis (NMKL) 1999b, Coagulase positive staphylococci. Enumeration in foods. 66 1999b, 3rd Ed.

Nordic Committee on Food Analysis (NMKL) 1999c, Salmonella. Detection in foods. 71 1999c, 5th Ed.

Nordic Committee on Food Analysis (NMKL) 2001a, Sampling and pre-treatment of foods and animal feedstuffs, for quantitative microbiological examination. 91 2001, 3rd Ed.

Nordic Committee on Food Analysis (NMKL) 2001b, Coliform bacteria. Detection in foods and fodder. 44 2001b, 5th Ed.

NRA, (National Renders Association) (2009). Fish and prions: meat and bone meal is safe for aquaculture feeds.

NRC (National Research Council), (1993). Nutrient Requirements of Fish. National Academy Press, Washington D.C., 114pp.

Pongmaneerat, J. and Watanabe, T. (1991) Nutritive value of protein of feed ingredients for carp Cyprinus carpio. Nippon Suisan Gakkaishi, 57, 503–510.

Robaina, L.; Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M. and Montero, D. (1997) Corn gluten meal and meat and bone meals as protein sources in diets for gilthead seabream *Sparus aurata*: nutritional and histological implications. Aquaculture, 157: 347–359.

SAS, (1991). SAS / STAT User's guide. SAS Institute, Cary, NC, USA, 1028.

Tacon, A.G.J. and Jackson, A.J. (1985). Utilization of conventional and unconventional protein sources in practical fish feeds. In Cowey, C.B.; Mackie, A .M. and Bell, J. G. (Eds.), Nutrition and Feeding in Fish Academe. Press, London. Pp 119-145.

USB, (2008). Nutrient specifications and quality standards for ingredients commonly used in diets for aquaculture species. US Soya bean Export Council.USA.

Wang, K.W.; Takeuchi, T., and Watanabe, T. (1985). Optimum protein and digestible energy level in diets for Tilapia nilotica. Bull. Jap. Soc. Sci. Fish, 51: 141-146.

Watanabe, T. and Pongmaneerat, J. (1991) Quality evaluation of some animal protein sources for rainbow trout Oncorhynchus mykiss. Nippon Suisan Gakkaishi, 57, 495–501.

Wu, Y.V.; Tudor, K.W., Brown, P.B. and Rosati, R.R. (1999) Substitution of plant protein or meat and bone meal in diets of Nile tilapia. N. Am. J. Aquacult., 61, 58–63.

Yamamoto, T.; Shima, T., Furuita, H. and Suzuki, N. (2002) Influence of feeding diets with and without fish meal by hand and by self-feeders on feed intake, growth and nutrient utilization of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 214, 289–305.

Yang, Y.; Xie, S., Cui, Y., Lei, W. Zhu, X., Yang, Y. and Yu, Y. (2004). Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp, *Carassius auratus gibelio*, Aquaculture Nutrition, 10: 289-294.

Yu, Y. (2004). Replacement of fish meal with poultry by-product meat and meat and bone meal in shrimp, tilapia and trout diets. www.uanl.mx/utilerias/nutriciou_acuicola/vii/archivos/qYuYu.PDF.

تأثير إستبدال مسحوق السمك بمسحوق اللحم والعظم في علائق البلطي النيلي
١- تقييم تغذوي وآمان إستخدامة

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تم إجراء تجربة نمو اولاً ، لإعادة تقييم تأثير إحلال مسحوق اللحم والعظم محل مسحوق السمك على أداء النمو والكفاءه الغذائيه في علائق أسماك البلطي النيلي. ثانيا ، لتقييم سلامة استخدام مسحوق اللحم والعظم بنسبة صفر، ٢٥، ٥٠، ٧٥ و ١٠٠% كمصدر بروتين حيواني في تغذية الأسماك. تم تركيب خمس علائق متزنه في محتواها من البروتين (٣٢%) و محتواها من الطاقه (٣٥٠٠ كيلو كالوري على اساس الطاقه المهضومه المفترضه)تحتوي على صفر ، ٣، ٦، ٩ و ١٢% مسحوق لحم وعظم . تم توزيع العلائق عشوائيا بحيث كانت كل عليقه تقدم لمجموعتين من الأسماك المتواجده في أحواض من الفيبر جلاس بحيث إحتوى كل حوض على عدد ٢٥ إصباعيه (متوسط وزن البدايه ١,٨ ± ٠,١٥ جم). وقد قدمت العلائق التجريبيه يدويا أربع مرات خلال اليوم لفترة ٦٠ يوماً. بالنسبه لتحكيم الأمان الميكروبي للأسماك المختبره، ثلاث عينات من كل مجموعه تم تجميعها في نهايه فترة التجربه في أكياس معقمه وذلك لقياس العد البكتيري الكلي،البكتريا المتحوصله، الباسيلس سيريس، سالمونيلا و الستافيلوكوكي .لم يلاحظ أي فارق معنوي من حيث وزن الجسم النهائي، الوزن المكتسب النسبي، معدل النمو النوعي بين الأسماك التي تغذت على العليقه القياسيه وتلك المغذاه على علائق إحتوت على مسوق لحم وعظم حتى مستوى إحلال ٥٠ % . بينما لم يلاحظ أي فارق معنوي من حيث كمية الغذاء المأكول ، معامل التحويل الغذائي و كفاءة البروتين النسبي بين الأسماك التي تغذت على العليقه القياسيه وتلك المغذاه على علائق إحتوت على مسوق لحم وعظم عند مستوى إحلال ٢٥ %. أقل أداء نتج عن مجموعة الأسماك التي تغذت على علائق إحتوت على ١٠٠% مسحوق لحم وعظم. في جميع العينات المختبره اظهرت عدم وجود خلايا كوليفورم حيه أو متحوصله، خلايا الباسيلس و السالمونيلا . بالنسبه لعد خلايا الستافيلوكوكي لم تكن هناك فروق معنويه بين مجاميع العينات المختبره. أوضحت الدراسه الأقتصاديه أن أفضل صافي إيرادات نتج عن إحلال ٢٥% من مسحوق سمك بمسحوق اللحم والعظم ٦٠,٩٢ جنيه تلاه العليقه القياسيه ٦٠,٨٨ جنيه ثم ٥٠% إحلال لمسحوق السمك بمسحوق اللحم والعظم ٥٨,٩٨ جنيه

هذه النتيجه أوضحت أنه يمكن إحلال ٥٠% من مسحوق السمك في علائق اسماك البلطي النيلي بمسحوق اللحم والعظم دون حدوث أي تأثير سلبي على أداء نمو أسماك البلطي النيلي أو سلامة إستخدامه. وإن كانت الدراسة الإقتصادية قد أوضحت ان نسبة إحلال ٢٥% هي الأحسن إقتصادياً. ويلزم إجراء دراسات تهدف لخفض محتوى الرماد في مسحوق اللحم والعظم ولتحسين معاملات الهضم بتحسين طُرق التصنيع.