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'L. CARNITINE MAY REDUCE THE HEPATOENTERIC ADVERSE EFFECTS OF FISH MEAL REPLACEMENT BY SOY BEAN IN *OREOCHROMIS NILOTICUS*

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SUMMARY

Soybean replacement of fish meal is known to induce adverse effects in the liver and the mucosal lining of the intestine. The aim of the present work was to investigate the possible ameleoration of these deleterious effects by addition of potassium dihydrogen phosphate and L.carnitine. For this purpose eighty, sex reversed male fish were used, they were allocated into four groups. G_1 control fed on a reference fish meal diet, G_2 (S30) in which soybean replace 30% of fish meal, G_3 soybean replace 30% of fish meal in addition to potassium dihydrogen phosphate KH_2PO_4 (8.42%) and G_4 as G_3 with addition of *L.carnitine* (150mg/kg diet).

The diet was prepared and provided to fish for 45 days, the initial and final body weight were recorded. At the end of the experiment blood samples were collected for determination of serum glucose, total protein, albumen, cholesterol, triglycerides, calcium, phospherous, estradiol-17β(E_2) and testosterone. Livers were weighed for calculation of HSI and histological sections from intestine and livers were prepared.

The results revealed that body weight was significantly decreased in G_2 and G_3. HSI was significantly decreased in G_3 and G_4. There was a marked increase in serum glucose level in G_4 as compared to other groups while serum total protein, cholesterol, triglycerides, Ca and P were significantly decreased in all groups as compared to control

Serum E_2 was significantly increased in G_2 but significantly decreased in G_4 as compared to

control. However G_4 showed a marked increase in serum testosterone level as compared to other groups .

Histological findings showed shortening in mucosal folds and reduction in absorptive vacuoles in middle intestine beside vacuolation in hepatocytes in fish fed soybean diet. These tissues nearly returned to its normal structure in G_4, which fed *L. carnitine* added to the diet.

It was concluded that partial replacement of fishmeal by soybean had an adverse effect on body weight, some serum metabolites, sex steroids and normal morphology of liver and intestine. The results recommend addition of *L. carnitine* to fish diets to improve body weight and reduce the adverse effects on hepatocytes and intestinal mucosa.

INTRODUCTION

Fishmeal has traditionally been a major ingredient in fish feeds because of its protein quality and palatability (Lovell, 1984). However, increasing demand, high cost and uncertain availability of fish meal (Barlow, 1989) have resulted in researchers studying alternative sources, particularly plant proteins to replace fish meal in the diet of a number of freshwater fish species (Dabrowski et al., 1989; Shiau et al., 1990 and Robinson and Li, 1994). Among plant proteins, soybean meal is the most promising candidate for partial or total replacement of fishmeal in fish diets. A considerable amount of fishmeal in the diet could be replaced with soybean meal in omnivorous freshwater species, such as carp, tilapia and catfish (Mohsen and Lovell, 1990 and Webster et al., 1992). Soybean has been recorded to contain considerable amounts of disadvantageous components, phytates and phytoestrogens (Eldridge, 1982 and NRC, 1993). Phytates interfere with phosphorus (P) absorption from fish gut by forming unabsorbable complexes. Two thirds of the soybean phosphorus is in the form of phytates, which has low availability in fish (Riche and Brown, 1993). In addition, presence of phytic acid in soybean meal seems to reduce the availability of zinc, this effect may be aggravated in the presence of excess dietary calcium (Galtin and Phillips, 1989). Thus, total replacement of fishmeal by various plant protein sources to reduce the P level in fish diet has resulted in poor growth (Kim et al., 1995). Under such conditions it is necessary to supplement the diets with an inorganic P source in order to meet the nutritional requirements. Phytoestrogens exhibit an estrogenic potency 1000 to 10,000 times less than estradiol-17β but its high concentration in the diet gave a strong estrogenic potency (Setchel, 1985). Any change from normal physiology is likely to be detrimental in some manner. Thus, the estrogenic contamination of male or immature fish by xenobiotic estrogenic compounds would have an impact on metabolism in these fish. This effect could have negative repercussions on growth, health and reproduc-

tion. Previous studies (Robaina et al., 1998) had indicated that phosphorus supplementation improves histological alteration induced by feeding diets containing soybean meal to gilthead seabream, Sparus aurata. Besides, subsequent transfer of fish from the soybean rich diet to a control (refrences diet) resulted in apparentiy normal intestinal mucosa after 3 weeks in Atlantic salmon, Salmo salar (Baeverfjord and Krogdahl, 1996). The effects of soybean on morphological and functional alterations in fish intestine were not fully studied in tilapia (*O.niloticus*) fed excess soybean as replacement fish meal. Accordingly, this work aimed to study the possibility of reducing the enteritis induced by soybean by addition of potassium dihydrogen phosphate and *L. carnitine*.

MATERIAL AND METHODS

Fish:

Eighty, sex reversed male Tilapia nilotica fish were transported from private fish farm to the laboratory in Faculty of Veterinary Medicine Kafr El-Sheikh. Fish were acclimated for two weeks and then allocated into four groups (20 fish of 17 + 2 g individual b.wt each) in, well aerated, thermostatically controlled temperature at 22°C, rectangular glass aquaria of 160 litre water capacity each.

Feeding: Fish were fed on diets as 5% of body weight for all groups. Diet composition was as in Table (1).

Table (1): Diet composition fed to Tilapia nilotica fish groups.

Group Number	Fish meal %	Soybean meal %	Corn %	Wheat %	Vitamins %	Mineral mixture %	KH_2PO_4
G_1 control	40	-	24	34	1.0	1.0	-
G_2 S_{30}	28	12	24	34	1.0	1.0	-
G_3 S_{30} + KH_2 PO_4	28	12	15.92	34	1.0	0.66	8.42
G_4 S_{30} + KH_2 PO_4 + *L. carnitine*	28	12	15.92	34	1.0	0.66	8.42

S_{30} = Soybean replace 30% of fishmeal

KH_2PO_4 = Potassium dihydrogen phosphate

L.carnitine was added to G_4 as 150mg/kg ration.

The diets were processed by blending the dry ingredients into a homogenous mixture, then made into paste by adding 30% water. The moist feed mixture was passed through a meat grinder, broken into pellets and dried in an air-forced oven at 60°C for 4 h to reduce the moisture to approximately 10 %.All feeds were packed in plastic bags and stored at 5C according to Boonyaratpalin et al. (1998).

Initial weight of fish was recorded after acclimatization period and then the fish were weighed weekly along the experimental period (45 days) to adjust the amount of feed provided to each fish group.

Sampling:

A. Blood samples:

Blood samples were collected from caudal blood vessels of each fish group at the end of the experiment. Serum samples were separated for biochemical and hormonal analysis.

1. Biochemical analysis:

Commercial kits from bioMerieux were used to measure colorimetrically serum glucose (Yeung, 2001), total protein (Peters, 1968), Albumin (Drupt, 1974), cholesterol (Trinder, 1969), Triglycerides (Jacobes and Van Demark, 1960), and ionized calcium (Gindler and King, 1972). Phosphorus was measured photometrically (Henry, 1964).

2. Hormone assay:

Serum estradiol-17β (E_2) (pg/ml) and testosterone (ng/ml) were measured by Enzyme linked-immunosorbent assay kits (ELISA) according to Raleliff and Carter (1988) and Odell and Swerdloff (1987).

B. Histological samples:

At the end of the experimental period, four fish were sampled for histological studies from each fish group. The abdominal cavity was opened along the ventral midline. The intestine was carefully dissected out, and a ring of approximately 1 cm in length was cut from the middle of this intestinal segment. The ring of intestine was cut open, and after carefully removing any intestinal contents, the sample was immersed in neutral phosphate-buffered formalin according to Baeverfjord and Krogdahl (1996). Livers were exteriorized, weighed to calculate hepatosomatic index (HSI) and then fixed in neutral phosphate buffered formalin. After fixation the samples were dehydrated in ethanol, equilibrated in xylene and embedded in paraffin according to routine techniques. Sections of approximately 5µ thickness were cut and stained with haematoxylin and eosin (H & E).

Statistical analysis: All data were subjected to statistical analysis according to Snedecor and Cochran (1980) using one way ANOVA test. Treatments were compared by the least significant difference test (LSD).

RESULTS

Table (2): Effect of partial replacement of fishmeal by soybean on final body weight and HSI of juvenile male Tilapia nilotica fish.

Groups Number	Initial body wt (g)	Final body weight (g)	HSI
G_1: control	19.15 ± 0.67^A	43.37 ± 0.37^A	0.021 ± 0.0008^A
G_2: S_{30}	19.07 ± 0.41^A	32.45 ± 0.39^D	0.018 ± 0.0005^B
G_3: $S_{30} + KH_2PO_4$	18.97 ± 0.80^A	37.42 ± 0.81^C	0.017 ± 0.0004^B
G_4: $S_{30} + KH_2PO_4$ + *L. carnitine*	19.25 ± 0.99^A	40.60 ± 0.88^B	0.016 ± 0.0003^B

S30 = Soybean replace 30% of fishmeal KH_2PO_4 added to the ration (8.42%)

L.carnitine added to the ration (150mg/kg). Values are mean + SE

Values with the same latter in each column are not significantly different at $P < 0.01$

Table (2) showed that, final body weight was significantly decreased in all fish fed soybean (30% of fish meal) as compared with control. *L carnitine* induced a significant increase in final body weight as compared to G_2. HSI was significantly ($P < 0.01$) decreased in all groups as compared to control.

There was a marked increase in serum glucose level in G_4 as compared to other groups. Meanwhile, serum total protein, cholesterol, triglyceride, calcium and phosphorus were significantly decreased in all groups as compared to control with a pronounced reduction there levels in G_4. Serum E_2 level was significantly increased there levels in G_2 but significantly decreased in G_4 as compared with control group. However, G_4 showed a marked increase in serum testosterone level as compared to other groups.

Table (3): Effect of partial replacement of fishmeal by soybean on some serum metabolites, Ca, P, E_2 and testosterone of juvenile male *Tilapia nilotica* fish.

Group Number	Glucose mg/dl	T. prot. g/dl	Albumin g/dl	Cholest. mg/dl	Triglyc. mg/dl	Ca mg/dl	P mg/dl	Estrogen E_2 pg/ml	Testosterone ng/ml
G_1 control	101.25^{C} ± 1.43	4.43^{A} ± 0.04	1.87^{B} ± 0.04	213.75^{A} ± 2.46	147.50^{A} ± 3.04	12.69^{A} ± 0.16	31.23^{A} ± 0.51	508.25^{C} ± 6.34	0.555^{C} ± 0.047
G_2 S_{30}	97.25^{C} ± 0.85	3.80^{B} ± 0.11	1.77^{BC} ± 0.09	181.00^{B} ± 8.37	128.0^{B} ± 3.26	10.73^{C} ± 0.12	20.41^{C} ± 0.39	1013.50^{A} ± 21.89	0.440^{D} ± 0.026
G_3 S_{30} + KH_2PO_4	106.75^{B} ± 1.65	4.00^{B} ± 0.09	2.12^{A} ± 0.04	182.00^{B} ± 4.32	130.50^{B} ± 4.40	11.12^{C} ± 0.06	23.29^{B} ± 0.52	721.75^{B} ± 11.9	0.668^{B} ± 0.022
G_4 S_{30} + KH_2PO_4 + *L. carnitine*	138.00^{A} ± 2.67	3.42^{C} ± 0.07	1.66^{C} ± 0.08	159.00^{C} ± 2.67	114.50^{C} ± 3.62	11.65^{B} ± 0.23	21.69^{C} ± 0.37	316.50^{D} ± 3.57	0.910^{A} ± 0.054

Values are mean + SE

Values with the same latter in each column are not significantly different at $P < 0.01$.

The histological examination showed that, the intestinal tissue of the fish fed on soybean (S30) had morphological changes represented in reduction of height of the intestinal folds (Fig. 2) as compared to the intestinal mucosa of fish fed on reference control diet (Fig. 1). Addition of the *L. carnitine* to soybean diet reduced these changes and tissues appears nearly like that of control.

Hepatocytes of G_2 showed vacuolation of cytoplasm (Fig. 4). *L. carnitine* group had nearly similar structure of hepatocytes to that of control group (Fig. 3).

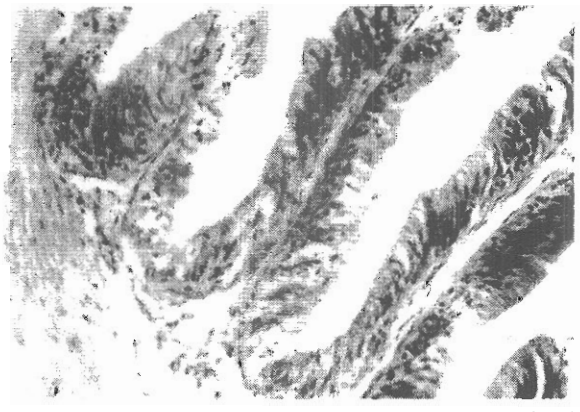

Fig. (1): The mucosal epithelium of intestine male tilapia fed control fish diet showing normal structure. Note well developed enterocytes, containing numerous absorptive vacuoles. (H & E x 100).

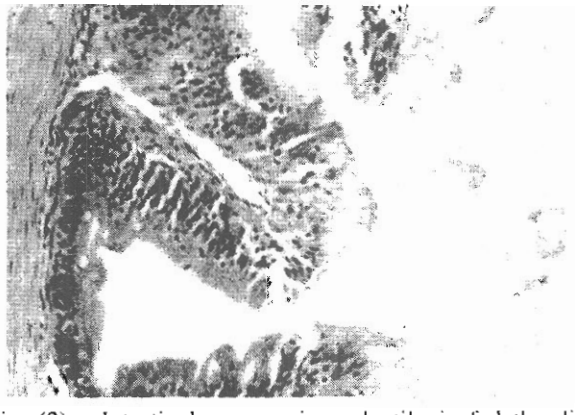

Fig. (2): Intestinal mucosa in male tilapia fed the diet S30 soybean meal showing reduction in the number of absorptive vacuoles in the mucosal epithelium of intestine. Microvilli are not clearly observed (H & E x 100).

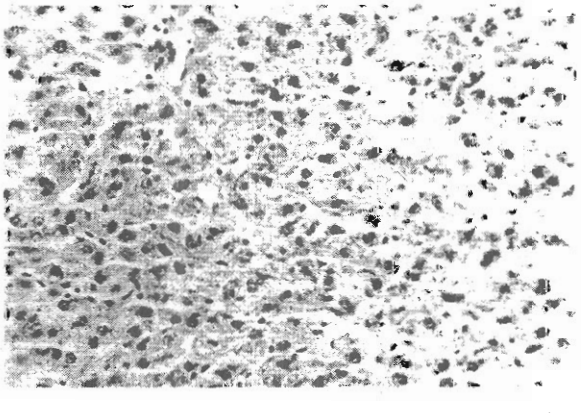

Fig. (3): Hepatocytes of *O. niloticus* fed on control fish meal diet showed normal structure and arrangement of hepatocytes (H & E x 100).

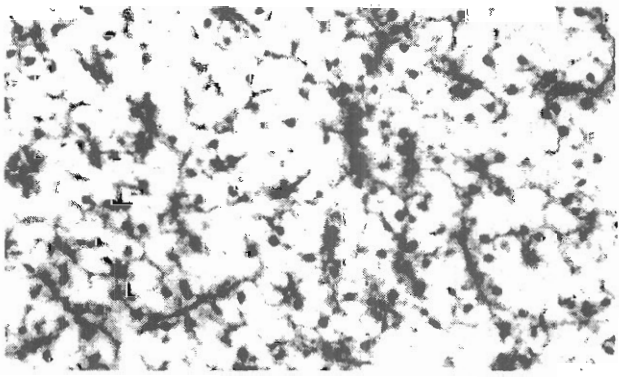

Fig. (4): Liver of *O. niloticus* fed on soybean S_{30} (G_2) showed vacuolation of hepatocytes. (H & E x 100).

DISCUSSION

Final body weight was significantly decreased in fish fed on soybean. This result was in agreement with Du and Niu (2003) who recorded that, specific growth rate and conversion efficiency decreased with increasing dietary soybean meal. Kikuchi (1999) found that, an increase of dietary soybean meal from 30% to 40% resulted in reduced growth and food utilization, inclusion of soybean meal as a substitute for fishmeal in the diet seemed to increase serum triglycerides and/or glucose concentration in fish.

Soybean contain phytic acid which affects the activation of trypsinogen and stability of trypsin that digest proteins of food (Caldwell, 1992). In addition, the phosphorus moiety of phytic acid is poorly available to fish and phytic acid forms low solubility complexes with divalent and trivalent ions (Budavar et al., 1989).

The growth rate was reduced significantly when 50% of the fishmeal was replaced by soybean meal introut diets (Alexis, 1990 and Booyaratpalin et al., 1998). In contrast, a considerable amount of fishmeal in the diet could be replaced by soybean meal in omnivorous freshwater fish, such as tilapia, carp and catfish (Mohsen and Lovell, 1990 and Webster et al., 1992). Robinson and Li (1994) also indicated that soybean meal could be used to completely replace fishmeal in the feeds for channel catfish reared in earthern ponds and fed daily to satiation. These contradictory results may be attributed to the differences in fish species, the percentage of soybean replaced, soybean treatment and/or the duration of feeding (Boonyaratpalin et al., 1998). Protein digestibility was negatively correlated with trypsin inhibitor activity. Unheated soybean meal had a high trypsin inhibitor activity. Feeding on soybean meal diminished growth rates, decreased intestinal trypsin activity and increased fecal excretion of protein and lipids in addition to the poor palatability of soybean meal (Berg Lea et al., 1989).

The problems related to use the soybean meal in fish diets have been ascribed to the presence of various antinutrient factors in soybean meal, among them lectins and protease inhibitors as well as a variety of allergic proteins (Liener, 1994). Of these, lectins have been shown to cause hyperplasia and hypertrophy in rat small intestine (Pusztai, 1989) and adverse effects on enterocyte viability (Ishiguro et al., 1992). The protease inhibitors have been shown to cause substantial decrease in protein digestibility in Atlantic salmon (Krogdahl and Olli, 1994). The histological findings of the present study (Fig. 2) in which a decrease in intestinal mucosal epithelium configuration and a change in the absorptive potency of enterocytes was agreed with the previous studies and explain the decrease in final weight of fish fed on soybean meal. Furthermore, the condition induced by dietary soybean meal in

distal intestine of salmon was classified as a non-infectious subacute enteritis, and a pathogenesis involving immunological mechanisms was also suggested (Baeverfjord and Krogdahl, 1996).

In the present work HSI was significantly decreased in fish fed soybean with or without L. carnitine. This result was in accordance with Robaina et al. (1998) who recorded that, a statistically lower values of HSI were observed in fish fed with diet (S30 + P). The fatty acid composition of lipid from soybean could affect liver lipid deposition in fish (Takeuchi et al., 1979). This was supported by the histoloigical finding in the present study in which vacuolation of cytoplasm of hepatocytes was observed (Fig. 4). Phosphorus supplementation significantly reduced HSI although it does not alter liver lipid deposition. Robaina et al. (1998) attributed this reduction to altered lipid and protein metabolism in fish fed with other diets which had lower P availability and subsequent higher liver lipid deposition, thus increasing HSI values.

In the present work there is a significant increase in serum glucose level with subsequent significant decrease in serum total protein, albumin, cholesterol and triglycerides in G_4 treated with L. carnitine accompanied with increase in final body weight. *L. carnitine* has a coenzyme function in the body. It acts as a vehicle for the transport of long chain fatty acids through the inner mitochondrial membrane to the site of beta oxidation, thereby promoting the oxidation of fatty acids (Groth et al., 1998). Santuli et al. (1990) showed an increase of the growth rate and an increase of protein content of the fry with increasing L. carnitine concentration in the diet. Burtle's research (1994) on the effect of *L. carnitine* supplementation in the diet of channel catfish resulted in a significant increase in growth, a reduction of lipid content in muscles and higher tolerance for ammonia stress, the findings in hepatocytes of G_4 in the present study were in concomitant with these results. *L. carnitine* leads to a protein-sparing effect, which is shown in increased protein levels besides it reduced lipid levels in tissues (Blum, 1993). It could be suggested that *L. carnitine* decreased triglyceride and cholesterol due to its entry as energy sources into mitochondria thus spare glucose which increase in level on expense of triglycerides and cholesterol. Replacement of fish oil by soybean oil resulted in a higher liver lipid deposition and lower peroxidation levels (Menoyo et al., 2004). Suslina (2003) found a hypoglycemic effect of *L.carnitine* in human. This contradictory results may be attributed to species variation or difference in dose, duration or age at treatment.

In the present study serum cholesterol, triglycerides, Ca and P were significantly reduced in fish fed soybean (S_{30}). These results are in consistence with Sam et al. (2003) and Hege et al. (2004) who recorded that, soyprotein significantly decreased plasma triglycerides, total choleste-

rol, low density lipid (LDL) cholesterol and high density lipid (HDL) cholesterol in rats. Besides, it reduced cholesterol accumulation in liver of hamster (Lin et al., 2004). Also, substitution of vegetable protein for animal protein in the diet using soy-based diets will lower cholesterol LDL and triglyceride levels (Anderson et al., 1995). The mechanism of lowering cholesterol and triglycerides levels is uncertain and may be due to phytoestrogens in soybean, which exerts a salutary effect on lipid profile similar to that of estrogens. Furthermore, Robert and Resental (2000) reported that, phytosterols in soybean compete with cholesterol for inclusion in mixed micelles, a necessary step for cholesterol absorption and so cholesterol level declines.

Calcium absorption was correlated with both serum E_2 and body weight in rats, hypoestrogenic status may be the cause of the decrease in calcium absorption in rats (Mariana et al., 2004).

Serum calcium and phosphorus was significantly decreased in fish fed on S30 diets in the present study (Table 3). These results agreed with Asgard and Shearer (1997) who recorded that, soy-concentrate-fed fish contained reduced whole body Ca, Mg and P. Inadequate uptake of P led to the inability of the fish to retain Ca and Mg. However, whole body Ca and retention of P were lower in fish fed the untreated soy concentrate compared to the phytase treated soy, whole body concentration of Ca was correlated to P independent on dietary treatment but the Ca: P was not constant (Storebakken et al., 1998).

The reduction in serum Ca and P of fish fed on soybean may be attributed to its phytate content which interfere with P absorption and make complexes with other divalent ions as Ca (Budavari et al., 1989).

Regarding serum E_2 in the present study, it is significantly increased in G_2 but significantly decreased in G_4 (Table 3). The marked increase of E_2 in G_2 was due to the phytoestrogens content of soybean (NRC, 1993). Phytoestrogens exhibit an estrogenic potency 1000 to 10,000 times less than E_2 but its high concentration in the diet gave a strong estrogenic potency (Stechel, 1985).

The estrogen receptor seems to have the lowest specificity among all the steroid receptors which have been characterized. This probably explains why the main effect observed as a sequence of steroid and steroid like containing contamination of the diet is an estrogenic one rather than androgenic or corticosteroidal one, even though diets contain steroids from all these groups (Pelissero et al., 1991a and Pelissero and Sumpter, 1992). Moreover, the estrogens have effects that are more noticeable than androgens, because the liver is one of the major target organs of these compounds. All estrogens or estrogen-like substances are able, once in the liver, to act as stimulating agents before, or instead of, being degraded. In

some cases it has even been argued that some of the enzymatic reactions in the liver can transform a weekly estrogenic compound into another which is more potent (Eisenfeld et al., 1980). *L. carnitine* was found to increase testosterone production in rats (Palmero et al., 1990) and in subfertile rams (Noseir and EL-Amrawi, 2001), this explain the increase in testosterone in G_4 supplemented with *L. carnitine* in the present study.

It was concluded that partial replacement of fishmeal by soybean had an adverse effect on body weight, some serum metabolites, sex steroids and normal morphology of liver and intestine. Addition of *L. carnitine* at the above mentioned level was needed to fish diets to improve body weight, lipid profile and reduce the adverse effects of soybean on intestinal and hepatic tissues.

REFERENCES

Alexis, M.N. (1990): Comparative evaluation of soybean meal as dietary ingredients for rainbow trout fingerling. Aquatic Living Resour. 3, 235-241.

Anderson, J.W.; Johnstone, B.M. and Cook-Newell, M.E. (1995): Meta-analysis of the effects of soy protein intake on serum lipid. N Engl J. Med., 333: 276-282.

Asgard, T. and Shearer, K. D. (1997): Dietary phosphorus requirements of Atlantic salmon (Salmo salar). Aquaculture Nutrition 3, 17-23

Baererfjord, G. and Krogdahl, A. (1996): Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: a comparison with intestine of fasted fish. J. Fish. Dis., 19: 375-387.

Baeverfjord, G. and Krgdahl, A. (1996): Development and regression of soybean meal induced enteritis in Atlantic salmon Salmo salar L., distal intestine: a comparison with intestines of fasted fish. J. Fish. Dis., 19: 375-387.

Barlow, S. (1989): Fish meal-world outlook to the year 2000. Fish Farmer, Sept./Oct., pp. 40-43.

Berg-Lea, T.; Brattas, L.E. and Krogdahl, A. (1989): Soybean proteinase inhibitor affect nutrient digestion in rainbow trout. In: Huisman, J. Van der Poel, T.F.B., Liener, I.E. (eds), Recent advances of Research in Antinutritional Factors in Legume Seeds. Pudoc, Wageningen, pp. 99-102.

Blum, R. (1993): *L. carnitine* in aquaculture. Presentation to LONZA LTD.

Boonyaratpalin, M.; Suranertanat, P. and Tunpibal, T. (1998): Replacement of fish meal with various types of soybean products in diets for Asian seabass "Lates calcarifer". Aquaculture, 161: 67-78.

Budavari, S.; O'Neil, M.J.; Smith, S. and Heckelman, P.E. (eds) (1989): The Merck Index. An Encyclopedia of chemicals, Drugs and Biologicals, 11th ed. Merck, Rehway, N.J., USA. pp. 1606.

Burtle, G.J. and Liu, Q. (1994): Dietary carnitine and lysine affect channel catfish lipid and protein composition. J. World Aquacult. Soc. 25(2): 169-174.

Caldwell, R.A. (1992): Effect of calcium and phytic acid on the activation of trypsinogen and the staof trypsin. J. Agric. Food Chem. 40: 43-47.

Dabrowski, K.; Poczyczynski, P.; Kock, G. and Berger, B. (1989): Effect of totally or partially replacing fishmeal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow

trout (Salmo gairdneri). New in vivo test for exocrine pancreatic secretion. Aquaculture. 77, 29-49.

Drupt, F. (1974): Determination of albumin in blood. Pharm. Biol., 9: 777.

Du, L. and Niu, C. (2003). Effects of dietary substitution of soya bean meal for fishmeal on consumption, growth, and metabolism of juvenile giant freshwater prawn, Macrobrachium rosenbergii. Aquaculture Nutrition. 9 (2): 139-143.

Eisenfeld, A.J.; Aten, R.F. and Dickson, R.B. (1980): Estrogen receptors in the mammalian liver. In: J.A. McLachlan (Editor), Estrogens in the Environment. Elservier North Holland, Amsterdam, pp. 69-96.

Eldridge, A.C. (1982): High performance liquid chromatography separation of soybean isoflavons and their glucosides. J. Chromatog., 234: 494-496.

Galtin, D.M. and Phillips, H.F. (1989): Dietary calcium, phytate and zinc interactions in channel catfish. Aquaculture, 79: 259-266.

Gindler, E.M. and King, J.B. (1972): Rapid colorimetric determination of calcium in biological fluids with methyl thymol blue. Am. J. Clin. Path., 58: 376.

Groth, A.; Focken, U.; Coloso, R. and Becker, K. (1998): Effects of L. carnitine on growth, survival and body composition of individually reared juvenile Tiger shrimp (Penaeus monodon). The 5th Asian Fisheries forum 11-14 Nov. Chiang, Mai Thailand.

Hege, W.; Liaset, B.; Oddrjum, A.; Einar, L and Roff, K.B. (2004): Fish protein hydrolysate reduces plasma total cholesterol, increased the proportion of HDL cholesterol, and lowers acyl-CoA: cholesterol acyltransferease activity in liver of Zucker rats. Amer. Soc. Nutr. Sci. J. 134: 1320-1327.

Henry, J.R.(1964): Clinical Chemistry, Harper and Row, Publishers, New York,. 415.

Ishiguro, M.; Nakashima, H.; Tanabe, S. and Sukakibara, R.(1992): Interaction of toxic lectins ricin with epithelial cells of rat small intestine in vitro. Chemistry and Pharmacology Bulletin, 40: 441-445.

Jacobes, N.J. and Van Demark, P.J. (1960): Biochemical analyses. Arch. Biochem. Biophys., 88: 250-255.

Kikuchi, K.(1999): Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (Paralichthys olivaceus). Aquaculture, 179: 3-11.

Kim, J.D.; Kim, K.S.; Song, J.S.; Woo, Y.B.; Jeng, K.S. and Wong, T.H. (1995): Effects of dietary full-fat soybean levels on growth performance and feed utilization and phosphorus excretion of carp (Cyprinus carpio). Asian-Austral J. Anim. Sci., 8: 587-594.

Kris-Etherton, P.M.; Harris, W.S. and Appel, L.J. (2003): Fish consumption, fish oil omega-3-fatty acid, and cardiovascular disease. Arterioscler thromb Vasc. Biol. 23: 20-31 AHA.

Krogdahl, A.; Lea, T.B. and Olli, J. (1994): Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilites in rainbow trout. Comp. Biochem. Physiol. 107A: 215-219.

Liener, I.E. (1994): Implications of antinutritional components in soybean foods. Critical Reviews in Food Science and Nutrition. 34: 31-67.

Lin, Y.; Gert, W.; Mario, A. and Elke, A. (2004): Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol fed hamsters. Amer. Soc. Nutr. Sci. 134: 143-148.

Lovell, R.J. (1984):Use of soybean products in diets for aquaculture species. Animal nutrition bulletin. Ameri-

cans Soybean Association February 1984, pp. 1-6.

Mariana, C.; Juan, P. and Sue, A.(2004): Estrogen prevents the reduction in fractional calcium absorption due to energy restriction in mature rats. J. Natr. 134: 1929-1934.

Menoyo, D.; Izquierdo, M.S.; Robaina, L.; Gines, R.; Lopez, C. and Baullsta, J. (2004): Adaptation of lipid metabolism, tissue composition and flesh quality in gelthead sea bream (Sparus aurata) to the replacement of dietary fish oil by linseed and soya bean oils. Br. J. Nutr., 92(1): 41-52.

Mohsen, A.A. and Lovell, R.T. (1990). Partial substitution of soybean meal with animal protein sources in diets for channel catfish. Aquaculture, 90: 303-311.

Noseir, W.M.B. and El-Amrawi,G. A. (2001): L carnitine supplementation to fertile and subfertile rams. 7th Intrnational Congress of Andrology. June 15-19, Montrial Quibec Canada, 409-413.

NRC (National Research Council) (1993): Nutrient requirements of fishes. National Academy Press, Washington D.C., pp. 114.

Odell, W.D. and Swerdloff, R.D.(1978): Abnomalities in gonadal function in men. Clin. Endoc., 8: 149-180.

Palmero, S.; Leone, M.; Prati, M., Cost, M.; Messeni, M.; Fgassa, E. and DeCecco, L. (1990): The effect of L-acetylcarnitine on some reproductive functions in the oligoasthenospermic rat Horm. Metab. Res. 22(12): 622-626.

Pelissero, C. and Sumpter, J.P. (1992): Steroids and "steroid-like" substances in fish diets. Aquaculture, 107: 283-301.

Pelissero, C.; LeMenn, F. and Kaushik, S. (1991a): Estrogens activity of soybean meal in the Siberian sturgeon (Acipenser baeri). Gen. Comp. Endocrinol., 83: 447-457.

Peters, T. (1968): Determination of total protein in blood. Clin. Chem. 14: 1147.

Puszati, A. (1989): Biological effects of dietary lectins. In: Recent Advances of Research in Antinutritional Factors in Legume Seeds (ed. by J. Huisman, T.F.B. Vander Poel + I.E. Liner). Proceeding from 1st International Workshop "Antinutritional Factors in Legume Seeds", 23-25, Wageningen, the Netherlands pp. 17-29.

Ralcliff, W. A. and Carter,C.D.(1988): Estradiol assay: application and guidelines for the provision of clinical biochemistry service. Ann. Clin. Biochem. 25: 466-483.

Riche, M. and Brown, P. B. (1993): Determination of phosphorus absorption coefficient for rainbow trout (Oncorhynchus mykiss) fed commercially used protein sources. Abstract of the World Aquaculture, 93, Torremolinos, Spain, May 26-28, 1993, p 258

Robaina, L.; Izquierdo, M.S.; Moyano, F.J.; Socorro, J.; Vergara, J.M. and Montero, D. (1998): Increase of dietary n-3/n-6 fatty acid ratio and addition of phosphorus improves liver histological alteration induced by feeding diets containing soybean meal to Gilthead seabream, Sparus aurata. Aquaculture, 161: 281-293.

Robert, L. and Rosenthal, M.D. (2000). Effectiveness of altering serum cholesterol levels without drugs. BUMC Proceeding. 13: 351-355.

Robinson, E.H. and Li, M.H. (1994): Use of plant proteins in catfish feeds. Replacement of soybean meal with cotton seed meal and replacement of fishmeal with soybean meal and cotton seed meal. J. World Aquacult. Soc., 25: 271-276.

Sam, J.B.; Ali, A.A.; Christian, H.; Patricia, L. and Tedine, R. (2003): Dietary flaxseed meal is more protective

than soy protein concentrate against hypertriglyceridemia and steatosis of the liver in an animal model of obesit. J. Amer. Coll. Nutr. 22(2): 157-169.

Santulli, A.; Puccia, E. and D'Ameliov, V. (1990): Preliminary study on the effect of short term carnitine treatment on nucleic acids and protein metabolism in sea bass fry. Aquaculture, 87: 85-99.

Setchel, K.D. (1985): Naturally occurring non-steroidal estrogens of dietary origin. In: J.A. McLachlan (Editor), Estrogens in the environment. Elsevier North Holland, Amsterdam. pp. 69-85.

Shiau, S.Y.; Lin, S.F.; Yu, S.L.; Lin, A.L. and Kwok, C.C. (1990): Defatted and full-fat soybean meal as partial replacement for fishmeal in tilapia (Oreochromis niloticus x O. aureus) diets at low protein level. Aquaculture, 86: 401-407.

Snedecor, G.M. and Cochran, W.G. (1980): Statistical analysis. 7th ed. (p. 215-237). Oxford and J.B.H. Publishing Company.

Storebakken, T.; Shearer, K.D. and Roem, A.J. (1998): Availability of protein phosphorus and other elements in fish meal, soy protein concentrate and phytase-treated soy-protein concentrate-based diets to Atlantic salmon (Salmo salar). Aquaculture, 161: 365-379.

Suslina, Z.A. (2003): Antioxidant activity of mildronate and L-carnitine in the treatment of patients with cerebrovascular diseases. EKSP. Klin. Farmakol. May-June 66(3): 32-35.

Takeuchi, L., Takeda, H. and Watanabe T. (1979): Availability of dietary phosphorus in carp and rainbow trout. Bull. Jap. Soc. Fish.,45: 1527-1532.

Trinder, P. (1969): Biochemical analyses. Ann. Clin. Biochem., 6: 24.

Webster, C.D.; Tidwell, J.H.; Goodgame, L.S.; Yancey, D.H. and Mackey, L. (1992). Use of soybean meal and distillers grains with soluble partial or total replacement of fishmeal in diets for channel catfish *Ictalurus punctatus*. Aquaculture, 106: 301-309.

Young, D.S. (2001): Effect of disease on clinical lab., tests 4th ed. AACC.