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# EFFECT OF DIETARY LEVELS OF LIPID AND CARBOHYDRATE ON GROWTH PERFORMANCE, CHEMICAL CONTENTS AND DIGESTIBILITY IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS* WALBAUM, 1792

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## ABSTRACT

The present study was carried out to determine the effects of four rations on growth, chemical composition and digestibility of the Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Four test diets were formulated to have the same levels of protein (40%) and energy (gross energy: around 4800 cal/g), however, these test diets were composed of basic diet with different carbohydrate (0, 3, 12 and 18%) to lipid (0, 6, 15 and 18%) ratios (Diet 1: 0/18; Diet 2: 3/15; Diet 3: 12/6 and Diet 4: 18/0). Fifty fish weighing 99.0 ± 0.65g were placed in each of the four experimental tanks and were fed with one of the experimental diets for 13 weeks consisting of two trial periods (Periods I and II). Growth parameters and chemical composition of fish flesh were monitored. Effects of the diets on apparent digestibility coefficients (ADC) were also determined. At the end of the study, the fish growth was affected significantly ($P<0.05$) with carbohydrate and lipid levels in the diets. Higher growth, better feed conversion ratio and protein efficiency ratio were observed in experimental groups fed with Diets 1 and 3. There were no significant ($P>0.05$) changes in specific growth rate, although higher values were observed in fish fed with Diets 1 and 3. The experimental group fed with Diet 4 showed a lower ($P<0.05$) growth and feed conversion ratio. The hepatosomatic index (HSI) showed no significant ($P>0.05$) changes in experimental groups fed with the diets. The percentage of water, protein and ash of fish flesh did not show any ($P>0.05$) change. However, the muscle lipid content of fish significantly ($P<0.05$) decreased as carbohydrate level increased. The ADC values were also affected ($P<0.05$) with carbohydrate and lipid levels in the diets. The ADC of lipid was lowest in the experimental group fed with Diet 1. The ADC values for dry matter, proteins and energy were lowest for fish fed on Diet 4, while for ash it was lowest for Diet 2. These results indicate that the best balance between the dietary lipid and carbohydrate levels was obtained on Diet 3, without lower growth.

**Key words:** *Oncorhynchus mykiss*, carbohydrate, lipid, growth, digestibility.

## INTRODUCTION

Fish diets containing high levels of proteins are necessary for the economic growth of fish in intensive rearing conditions (De-Silva and Anderson, 1998). Ratios of protein levels of fish feeds generally vary from 25 to 60% and for the salmonid feeds these ratios are 40-50% (Murai, 1992). High protein content of the diet is essential for the growth of carnivorous fish. However, protein is an expensive component of diet. If trout are cultured intensively, feed costs may increase due to higher dependency on artificial feeding. Therefore, the expensive protein portion of the diet should be optimally utilized for growth rather than maintenance. Since protein is an expensive component of the fish diet, the optimization of protein level in diet is necessary. Therefore, inclusion of appropriate levels of non-protein energy sources in the diet determining the efficiency of protein utilization is important (Wilson, 1994). Carbohydrates and lipids are cheaper non-protein energy sources compared to proteins. Optimal level of protein and the protein-sparing effect of non-protein nutrients such as lipids or carbohydrates may be effective in reducing feed costs.

The protein sparing effect of lipids has been shown to be effective in several fish species (De-Silva and Anderson, 1998; Sargent and Tacon, 1999). However, commercial diets containing high or low levels of lipids may affect fish growth negatively and may result in fatty fish. Fat-deficient diets, on the other hand, may result in growth retardation and physiological symptoms (De-Silva and Anderson, 1998).

The effect of dietary carbohydrate on fish growth seems to depend on the source, dietary concentration and digestibility, the level of dietary intake, rearing conditions and fish species (Hilton and Atkinson, 1982; Kim and Kaushik, 1992; Krogdahl *et al.,* 2005). The protein-sparing effect of different sources and levels of carbohydrates has been debated (Hilton and Atkinson, 1982; Wilson, 1994; Stone, 2003). Hilton and Atkinson (1982) reported that high dietary levels (>14%) of cereals depressed growth of rainbow trout. However, it has been demonstrated that digestible carbohydrates, such as gelatinized or extruded starch, improve protein and energy utilization in the same species (Medale *et al.,* 1991; Kim and Kaushik, 1992; Medale *et al.,* 1994). The aim of this study was to evaluate the effect of four dietary inclusion levels of gelatinized starch and lipids on growth performance, nutrient utilization and body composition of rainbow trout reared in freshwater.

## MATERIALS AND METHODS

### Fish and rearing conditions

The experiment, consisting of two growth periods of 13 weeks each (Periods I and II), was carried out at Gözde Fish Farming, Yeşilbaş, Ağlasun, Burdur, Turkey, from 1 May to 31 October, 2001. The trial was conducted on groups of 50 fish (with an average initial weight of 99g) each in circular fibreglass tanks (water volume 400 liters) with a flow-through water system. Water temperature was kept between 14 and 16$^{o}$C and tanks were kept under natural photoperiod. Four diets were randomly assigned to groups of fish. The commercial basic diet was prepared by Korkutelim Yem San Tic. A.Ş., Antalya, Turkey and experimental diets were prepared by adding the necessary ingredients to the basic diet. Carbohydrate/lipid was added in the following proportions: Diet 1:0/18; Diet 2: 3/15; Diet 3: 12/6 and Diet 4: 18/0 (Table 1). After mixing, food was prepared in pellet form. The amount of daily diet for the experimental groups was the 2.0% of the mean body weight of the fish. Groups were fed with the experimental diets twice a day.

### Growth studies

The first trial lasted 13 weeks and after that a second trial was also carried out for another 13 weeks. At the beginning of experiments and every 30 days, all fish from each tank were removed, anesthetized using quinaldine (1/20.000), weighed and then returned to their corresponding tanks. In addition, a random sample of five fish was removed from each tank, killed for carcass composition analysis at the beginning and at the end of the trial, and then immediately frozen at –20$^{o}$C until analysis. At the end of the experiment, five fish were also randomly selected, their livers were removed and weighed for hepatosomatic index (HSI) calculation.

### Digestibility studies

At the end of the experiment, digestibility coefficients were determined by feeding with diets containing $Cr_2O_3$ as a marker for three weeks. Faeces from each group were collected every day by the stripping method. Pooled faeces from each treatment group were homogenized and stored at –20$^{o}$C. Apparent digestibility coefficients (ADC) of diet composition were calculated as:

$ADC = 100-[100\ (\%M_F/\%M_D)\ (\%N_D/\%N_F)]$, where M and N are marker and nutrient concentrations; subscripts D and F represent diet and faeces, respectively (Maynard and Loosli, 1969).

### Chemical analysis

Proximate diet composition, chemical composition of the faeces and fish were determined using the following procedures: dry matter content by drying in an oven at 105$^{o}$C for 24 h; crude protein (as g Nx 6.25) by the Kjeldahl method after acid digestion; fat by the soxhlet method after ethylether extraction; ash by combustion at 550$^{o}$C in a muffle furnace for 24 h; crude cellulose after an alkali and acid digestion; gross energy with an adiabatic bomb calorimeter (Gallenkamp Autobomb) calibrated with benzoic acid (AOAC, 1995); $Cr_2O_3$ in the diets and faeces contents were measured using a spectrophotometr involving perchloric acid digestion (Furukawa and Tsukahara, 1966).

### Statistical analysis

Results are expressed as mean (± SD). Statistical tests were carried out on transformed data, because of non-homogenous variances. Differences between dietary treatments were determined by analysis of variance. Differences between means were calculated using Duncan's multiple range test at a significance level of $P<0.05$. All statistical tests were performed using the SAS package (Sas Institute, 1987).

## RESULTS

Changes in the mean body weight of rainbow trout reared on four experimental diets are shown in Table 2. Initial body weight did not differ among treatments, but final body weight varied significantly with varying carbohydrate and lipid levels. Highest growth was observed in fish fed with Diet 1 and 3. Fish fed with the highest carbohydrate level (Diet 4) tended to grow slower ($P<0.05$). The mean specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of each group of trout are given in Table 2. The poorest FCR and PER were for fish fed with Diet 4. The best FCR and PER were observed with Diets 1 and 3. At the end of the experiment, the SGR and hepatosomatic index (HSI) did not differ between treatments, but the highest value of HSI was obtained in fish fed on diet with the higher carbohydrate level (Table 2), while reverse was true for SGR.

Body composition values of fish fed different carbohydrate and lipid levels are given in Table 3. Significantly ($P<0.05$) higher content of carcass lipid was observed in fish fed with Diet 1 compared to those fed with the other diets. The content of protein, water and ash of fish carcass was slightly changed among the four dietary treatments, although there were no significant differences among treatments.

The apparent digestibility coefficients of the experimental diets are given in Table 4. Experimental diets had a significant effect on ADC ($P<0.05$). As expected, the crude protein digestibility was higher for Diet 2 than for the other diets. In general, apparent digestibilities increased with decreased levels of carbohydrates in the diet and increased levels of lipids. However, lipids digestibility increased significantly with decreasing dietary lipid contents and increasing dietary carbohydrate contents. The dry matter digestibility was lower in Diet 4 than the other diets. The digestibility of energy was the highest in fish fed with Diet 2 and lowest in fish kept on Diet 4.

## DISCUSSION

The results of the present study clearly indicate that the growth rate of trout was affected by the dietary levels of non-protein energy ($P<0.05$). Fish fed Diets 1 and 3 showed higher final weight. Growth was reduced when the carbohydrate content of the diet was increased, suggesting that the high carbohydrate levels led to poorer weight gains. Beamish and Medland (1986) reported protein-sparing effect in rainbow trout when lipid content was increased from 12 to 24%. However, Mazur *et al.* (1992) found that the diet containing 28.7% of gelatinized starch had an adverse effect on growth of chinook salmon. The results of this study indicated that the final weight and growth parameters were improved when the fish were fed with Diet 1 with high-lipid level, whereas Diet 4 containing high-carbohydrate level did not show the same effect. In addition, reduction in dietary lipid level (Diet 2) with concomitant increase in carbohydrate level (Diet 3) significantly improved the growth in rainbow trout. Similar results have been reported by Kaushik and Oliva-Teles (1985) and Brauge *et al.* (1994). Brauge *et al.* (1994) suggested that 30% of carbohydrate is the upper limit for rainbow trout diets. Findings of the present study are in agreement with the suggestions of Hilton and Atkinson (1982), Wilson (1994) and Medale *et al.* (1994), indicating that the optimal dietary level of digestible carbohydrates for rainbow trout is lower than 30%.

FCR and PER were influenced by dietary treatment. Better FCR was obtained by Diet 1. FCR and PER of trout were also improved slightly when they were fed with Diet 3. FCR and PER were negatively influenced with increasing carbohydrate level in diets. Similar results were reported in previous studies in which rainbow trout were fed with diets characterised by different levels of carbohydrates and lipids (Medale *et al.,* 1991; Kim and Kaushik, 1992; Wilson, 1994; Brauge *et al.,* 1994).

It is well known that liver size is directly related to hepatic glycogen level in salmonids (Kim and Kaushik, 1992). Absorbed carbohydrate which is utilized to provide energy can be deposited in the liver both as glycogen and as lipid after conversion. The HSI value was not changed with the diets containing lipid and carbohydrate. However, HSI was slightly higher for fish fed with high carbohydrate level diets. This is in agreement with the suggestions of Hilton and Atkinson (1982), Kaushik and Oliva-Teles (1985), Mazur *et al.* (1992), Kim and Kaushik (1992) and Brauge *et al.* (1994).

The effect of carbohydrate and lipid levels on body composition (lipid, protein, ash and water) of rainbow trout has been investigated by a number of researchers (Kaushik and Oliva-Teles, 1985; Brauge *et al.,* 1994). The lipid composition of the fish body was influenced by the dietary carbohydrate and lipid levels. The lipid content of muscle was higher in fish fed with the high lipid level diet. These increments of body lipid by increasing dietary lipid level have been reported in previous studies (Kaushik and Oliva-Teles, 1985; Medale *et al.,* 1991). A similar relationship between dietary carbohydrate level and whole body lipid content exists. The increase in dietary carbohydrates results in a decrease in body lipid content (Hilton and Atkinson, 1982). This effect can be due to the poor ability of rainbow trout to digest carbohydrates, resulting in lower weight achieved with these diets. The results of the present study indicate that high levels of dietary carbohydrate yielded no particular benefit in improving the performances and muscle composition of rainbow trout. Dietary lipid level, on the contrary, plays an important role in influencing growth rate and muscle composition of this species.

The negative relationship between dietary carbohydrate content and digestibility is in agreement with previous studies with salmonids fed on digestible complex carbohydrates (Aksnes, 1995; Hemre *et al.,* 1995; Storebakken *et al.,*1998; Stone, 2003; Krogdahl *et al.,* 2005). However, in some of the studies, the effects of dietary lipid level on lipid digestibility were not found (Kim and Kaushik, 1992; Brauge *et al.,* 1994). Crude ash and lipid digestibility increased with decreasing lipid level and increasing carbohydrate level. The digestibility of protein and dry matter increased with decreasing level of carbohydrates and increasing level of lipids. These findings are in agreement with the suggestions of Kim and Kaushik (1992) and Brauge *et al.* (1994). The apparent digestibility of energy was influenced positively with concomitant carbohydrate and lipid levels, which is in agreement with the suggestion of Brauge *et al.* (1994). The apparent digestibility coefficients of the fish diet can also be affected by the protein level in the diet (Gul *et al*., 2007).

**Table 1: Composition of the experimental diets[a]**

| Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| Basic diet (%)[b] | 82 | 82 | 82 | 82 |
| Gelatinized corn starch (%) | 0 | 3 | 12 | 18 |
| Lipid (fish oil) (%) | 18 | 15 | 6 | 0 |
| **Proximate composition of diets (%)** | | | | |
| Crude protein | 40.03 ± 0.205 | 40.35 ± 0.020 | 39.81 ± 0.260 | 40.12 ± 0.320 |
| Lipid | 24.69 ± 0.905 | 20.65 ± 0.240 | 11.13 ± 0.680 | 4.950 ± 0.450 |
| NFE[c] | 15.39 ± 1.350 | 18.59 ± 0.850 | 28.56 ± 0.940 | 33.72 ± 1.452 |
| Ash | 11.20 ± 0.060 | 11.79 ± 0.010 | 10.70 ± 0.180 | 10.95 ± 0.650 |
| Moisture | 5.307 ± 0.030 | 5.126 ± 0.340 | 6.320 ± 0.670 | 6.740 ± 0.188 |
| Cellulose | 2.050 ± 0.040 | 2.500 ± 0.082 | 2.400 ± 0.560 | 2.150 ± 0.290 |
| Gross energy (cal/g) | 5175.3 ± 1.850 | 4915.0 ± 3.010 | 4803.1 ± 2.420 | 4775.9 ± 4.680 |
| $Cr_2O_3$ | 0.980 ± 0.001 | 0.990 ± 0.005 | 0.970 ± 0.010 | 0.998 ± 0.002 |

[a]Values are means (± SD) of three replicates.
[b]Basic ingredients : Fish meal (65), 44.08; Soybean, 16.19; Bonkalit, 11.46; Corn gluten, 4.84; Meat-Bone, 4.38; Vitamin, 0.44; Mineral, 0.18; Pellet binder, 0.35; Antioxidant, 0.02.
[c]Nitrogen-free extract.

**Table 2: Growth performance in trout fed the experimental diets[1]**

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| Initial wt. (g/fish) | 99.23 ± 0.540 | 99.01 ± 0.230 | 99.41 ± 1.050 | 99.01 ± 0.680 |
| Final wt. (g/fish) | 198.0 ± 0.910 $^{a}$ | 180.2 ± 1.555 $^{b}$ | 188.9 ± 2.440 $^{ab}$ | 168.8 ± 0.850 $^{c}$ |
| SGR ($day^{-1}$%)[2] | 0.928 ± 0.160 $^{a}$ | 0.888 ± 0.120 $^{a}$ | 0.900 ± 0.156 $^{a}$ | 0.735 ± 0.005 $^{a}$ |
| FCR[3] | 1.927 ± 0.340 $^{a}$ | 2.260 ± 0.826 $^{ab}$ | 2.190 ± 0.903 $^{a}$ | 2.610 ± 0.558 $^{b}$ |
| PER[4] | 1.420 ± 0.220 $^{a}$ | 1.110 ± 0.165 $^{ab}$ | 1.254 ± 0.278 $^{ab}$ | 0.900 ± 0.156 $^{b}$ |
| HSI (%)[5] | 1.151 ± 0.072 $^{a}$ | 1.133 ± 0.080 $^{a}$ | 1.270 ± 0.087 $^{a}$ | 1.522 ± 0.112 $^{a}$ |

[1]Each value is the mean (±SD) of three replicates. Values in a row with different superscripts are significantly different from each other (P<0.05).
[2] Specific growth rate (SGR) = [In final mean body wt. (g)] - [In initial mean body wt. (g)]/days x100
[3] Feed conversion ratio (FCR) = g feed consumption/ (g final biomass –initial biomass).
[4] Protein efficiency ratio (PER) = dry body wt. (g)/protein intake (g)
[5] Hepatosomatic index (HSI) = [wet liver wt.(g)x100]/wet body wt. (g).

**Table 3: Body composition of trout fed with experimental diets[1]**

| | Fish body composition (%) | | | |
|---|---|---|---|---|
| Diets | Water | Protein | Lipid | Ash |
| Initial fish | 77.2 ± 0.46 | 18.1 ± 0.10 | 2.61 ± 0.41 | 1.44 ± 0.02 |
| Diet 1 | 74.2 ± 0.60 $^{a}$ | 19.2 ± 0.86 $^{a}$ | 4.70 ± 0.14 $^{a}$ | 1.36 ± 0.05 $^{a}$ |
| Diet 2 | 75.8 ± 0.52 $^{a}$ | 18.8 ± 0.06 $^{a}$ | 3.29 ± 0.30 $^{b}$ | 1.35 ± 0.08 $^{a}$ |
| Diet 3 | 76.1 ± 0.21 $^{a}$ | 18.5 ± 0.01 $^{a}$ | 3.41 ± 0.44 $^{b}$ | 1.31 ± 0.11 $^{a}$ |
| Diet 4 | 76.1 ± 0.70 $^{a}$ | 18.7 ± 0.76 $^{a}$ | 3.05 ± 1.00 $^{b}$ | 1.37 ± 0.01 $^{a}$ |

[1] Each value is the mean (± SD) of three replicates. Values in the same column not sharing the same superscripts are significantly different (P<0.05).

**Table 4: Effects of different diets on apparent digestibility coefficients (ADC) of nutrients[1]**

| | ADC (%) | | | | |
|---|---|---|---|---|---|
| Diets | Dry matter | Ash | Protein | Lipid | Energy |
| Diet 1 | 55.36 ± 0.09 $^{a}$ | 6.120 ± 0.51 $^{bc}$ | 65.22 ± 0.65 $^{b}$ | 83.77 ± 0.35 $^{d}$ | 63.19 ± 0.48 $^{b}$ |
| Diet 2 | 58.15 ± 1.12 $^{a}$ | 4.410 ± 1.48 $^{c}$ | 70.01 ± 0.17 $^{a}$ | 88.66 ± 1.13 $^{b}$ | 64.82 ± 0.04 $^{a}$ |
| Diet 3 | 55.44 ± 0.02 $^{a}$ | 16.85 ± 2.00 $^{a}$ | 63.56 ± 0.32 $^{b}$ | 87.46 ± 0.18 $^{c}$ | 63.83 ± 0.07 $^{b}$ |
| Diet 4 | 52.72 ± 0.54 $^{b}$ | 10.50 ± 0.94 $^{a}$ | 62.41 ± 1.06 $^{b}$ | 89.56 ± 0.81 $^{a}$ | 58.84 ± 0.03 $^{c}$ |

[1]Each value is the mean (± SD) of three replicates. Values in the same column not sharing the same superscripts are significantly different (P<0.05).

In the present study, final body weights were not significantly different (P>0.05) in fish fed on Diets 1 and 3. Lipid is an expensive non-protein energy source in a diet and diet costs may increase due to higher dependency on commercial diets. In addition, high dietary lipid levels resulted in a significantly increased lipid level in fish and decreased nutrient digestibility. Based on growth performance, body composition, nutrient digestibility and cheaper non-protein energy source of carbohydrate, Diet 3 seems optimal for rainbow trout. However, higher dietary lipid may affect negatively the body composition and nutrient digestibility. Moreover, higher lipid levels are also an expensive non-protein energy source in diets. On the other hand, higher dietary carbohydrate with low lipid or low carbohydrate with high lipid levels may result in lower growth and poor nutrient digestibility and body composition of fish.

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## REFERENCES

Aksnes, A., 1995. Geowth, feed efficiency and slaughter quality of salmon, *Salmo salar* L., given feeds with different ratios of carbohydrate and protein. Aquacult. Nutr., 1: 241-248.

AOAC, 1995. Offical methods of analysis. 16th Ed., Association of Offical Analitical Chemists, Arlington,VA, USA.

Beamish, F. W. H. and T. E. Medland, 1986. Protein sparing effects in large rainbow trout, *Salmo gairdneri*. Aquaculture, 55: 35–42.

Brauge, C., F. Medale and G. Corraze, 1994. Effect of dietary carbohydrate levels on growth, body composition and glycaemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. Aquaculture, 123: 109-120.

De-Silva, S. S. and T. A. Anderson, 1998. Fish Nutrition in Aquaculture. Chapman and Hall, London, UK.

Furukawa, A. and H. Tsukahara, 1966. On the acid digestion method for determination of chromic oxide as an indicator substance in the study of digestibility in fish. Bull. Jap. Soc. Sci. Fish., 32: 502-506.

Gul, Y., M. Salim and B. Rabbani, 2007. Evaluation of apparent digestibility coefficients of different dietary protein levels with and without fish meal for *Labeo rohita*. Pakistan Vet. J., 27(3): 121-125.

Hemre, G. I., K. Sonders, O. Torrissen and R. Waogebo, 1995. Carbohydrate nutrition in Atlantic salmon (*Salmo salar* L.,): Growth and feed utilization. Aquacult. Fish. Res., 26: 149-154.

Hilton, J. W. and J. L. Atkinson, 1982. Response of rainbow trout *Salmo gairdneri* to increased levels of available carbohydrate in practical trout diets. British J. Nutr., 47: 597-607.

Kaushik, S. J. and A. Oliva-Teles, 1985. Effects of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture, 50: 89-101.

Kim J. D. and S. J. Kaushik, 1992. Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 106: 161-169.

Krogdahl, A., G. I. Hemre and T. P. Mommsen, 2005. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. Aquac. Nutr., 11: 103-122.

Maynard, L. A. and J. K. Loosli, 1969. Animal Nutrition. 6th Ed., McGraw-Hill, New York, USA.

Mazur, C. N., D. A. Higgs, E. Plisetskaya and B. E. March, 1992. Utilization of dietary starch and glucose tolerance in juvenile Chinook salmon *Oncorhynchus tshawytscha* of different strains in seawater. Fish Physiol. Biochem., 10: 303–313.

Medale, F., P. Aguirre and S. J. Kaushik, 1991. Utilization of dietary carbohydrates by rainbow trout at two water temperatures. In: Wenk, C., Boessinger, M. (eds), Energy Metabolism of Farm Animals. Proc. 12th Symp. Energy Metabolism of Farm Animals, Kartause Ittingen, Switzerland, 1–7 September 1991. Eaap Pub., 58: 392–395.

Medale, F., C. Brauge and G. Corraze, 1994. Effect of dietary non-protein energy source on substrate oxidation and lipogenesis in rainbow trout. In: Aguilea, J.F. (ed.), Energy Metabolism of Farm Animals. Proc. of the 13th Symp. Tojaca (Esp.), 1994/09/18-24. (Eaap Publication No: 76) Csıc, Granada (Esp.)., 133-137.

Murai, T., 1992. Protein nutrition of rainbow trout. Aquaculture, 100: 191-207.

Sargent, J. R. and A. G. J. Tacon, 1999. Development of farmed fish: a nutritionally necessary alternative to meat. Proc. Nutr. Soc., 58: 377-383.

Sas Institute, 1987. Sas User's Guide. Release 6.03 Ed., Cary, North Caroline, Sas Institute Inc, USA.

Stone, D. A. J., 2003. Dietary carbohydrate utilization by fish. Rev. Fish. Sci., 11: 337-369.

Storebakken, T., K. D. Shearer, S. Refstie, S. Lagocki and J. Mccool, 1998. Interactions between salinity, dietary carbohydrate source and carbohydrate concentration on the digestibility of macronutrients and energy in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 163: 347-359.

Wilson, R. P., 1994. Utilization of dietary carbohydrate by fish. Aquaculture, 124: 67-80.