VITAMIN AND ESSENTIAL FATTY ACID SUPPLEMENTATION IN EXPERIMENTAL DIETS FOR RAINBOW TROUT (Salmo gairdneri, R.)

By

SOFRONIOS E. PAROUTICOLOU

Institute of Oceanographic and Fisheries Research

Athens - Greece

ABSTRACT

Four isocaloric experimental diets, each containing different amounts of vitamins and essential fatty acids, were fed to four groups of rainbow trout fry for 8 weeks. The growth rate, food conversion factor, mortality, the amounts of liver fat and glycogen, the hematocrit values and the biochemical composition of the major fractions on carcass samples - crude protein (N x 6.25), crude fat, ash and moisture - of these groups of fish have been determined. The use of reduced amounts of the source of essential fatty acids, as well as of the nucleic acid, vitamin E, vitamin K₂, inositol and choline levels, in the formulation of one of the experimental tested menus, supported sufficient growth rate, protein efficiency ratio and food conversion factor. The absence of dry defatted milk or rice from one diet caused the accumulation of the highest liver glycogen level and the lowest fat content, while the combination of various nutrients in one of the used menus gave the highest carcass protein level and hematocrit value. The possibility of identifying simple to prepare, and sufficiently economical and nutritional dry trout diets, by using a proper vitamin essential fatty acid premix in natural ingredient combination has been investigated.

1. INTRODUCTION

The requirements of rainbow trout and salmon for water and fat-soluble vitamins have been demonstrated (HALVER, 1957, COATES and HALVER, 1958, HALVER, 1972). Also, the essential fatty acid (E.F.A.)
requirements of rainbow trout have been reported by Sinhuber et al., (1969, 1972), Yu and Sinhuber (1973).

Taking into consideration the fact that the qualitative and quantitative identification of the vitamin and E.A. for salmonoids, has been achieved mostly by using pure sources of protein, fats, carbohydrates and minerals, it could be noted that the proposed amounts of vitamins and E.A. identified as required by salmonoids could be changed when artificial dry diets are prepared by combinations of natural products or and by-products.

However, many reports which are dealing with trout and salmon nutrition with synthetic or semisynthetic diets, emphasize the economical benefit which is produced by several combinations or replacements of the proportions of various natural ingredients in the test diets using a constant vitamin and E.A. premix (Follier et al., 1964, Follier and Bann, 1967, Cho et al., 1974, Rumsey and Keidla, 1974), while, other investigators have achieved remarkable results in this field, working on the determination of the amount of natural fat and replacement of cereals with suitable protein sources in the diets, using similarly, a constant vitamin mixture (Bergstrom, 1973).

The purpose of this experiment, which is a part of related investigations on fish nutrition being carried out at this laboratory, is to determine the most suitable mixture of vitamins and E.A. in constant ingredients - combined experimental dry diets, composed mainly of various local protein, fat and carbohydrate sources, in order to produce nutritionally and economically sufficient diets for the artificial propagation of rainbow trout.

2. MATERIAL AND METHODS

Four groups of 45 days old rainbow trout (Salmo gairdneri) were used as test fish in this experiment. Each group consisted of 10 fishes (mean initial weight 83 g.), hatched from the National Hatchery Station of Edessa and fed previously on a commercial trout diet.

The fish were maintained in experimental 150 l closed system aquaria using dechlorinated city water which was continuously recycled and entirely regenerated three times weekly. Water temperature was 13°C±1°C and its dissolved oxygen content 10ppm.

Fish were fed by hand five times a day, six days weekly. Amounts of food given and mortality were recorded daily. Dead fish were not replaced. The entire population of each aquarium was weighed every fortnight through the experiment and the amount of food was adjusted according to the weight of the fish.

By the end of the experiment the hematocrit values of 10 fishes. (4
samples from each fish) from each group population, were obtained by
severing the caudal peduncle and using heparinized precalibrated
capillary tubas and Clay Adams, readacrit centrifuge. Individual deter-
minations of liver glycerogen levels of 16 fish, taken randomly from each
diet group were made. Also, the livers of 10 fish from each diet were
obtained for the determination of their total fat content. The other fish
from each population were minced, homogenized and after lypophiliza-
tion were used for protein, fat and ash determinations. Prior to the
preparation of diets all ingredients were individually ground and the
diets' formulations were based on chemical analysis of the feeds.
The analytical methods used for feeds and fish samples are as follows:
Moisture. 10 samples of each material drying to constant weight,
usually 24 hr at 70°C; carcass moisture determinations of the fish sam-
ple were undertaken before lypophilization; protein (Nx6.25), 18 sam-
ple of each material by Kjeldahl procedure; fat, petroleum ether 40°-
60°C extractions of 7 samples; liver fat and glycerogen contents were
determined by the method of FOLCH et al. (1957) and MONTGOMERY
(1957) respectively; and ash, 10 samples of each material, ignited to
constant weight, usually 12 hr in 500°C. The qualitative and quan-
titative determination of amino acids of some of the ingredients used
was carried out with the use of a Technicon Auto amino acid analyser.
All diets were ground using a common mixing apparatus. The diets
were usually prepared to 1 week to 10 days amounts and were held in
the refrigerator (5° - 10°C).
As one of the main reasons in the carrying out of this experiment
is the definition of several combinations of different ingredients and
vitamin E.F.A. premixtures for artificial nutrition of trout with
economic interest, the full formulae of the tested diets are not
reported in this paper. However, the main protein source of G, H and I
diets is composed of a combination of animal products including dry
defatted silk-worm pupae, which is not contained in the formulation of
diet J. The basic difference between G, H and I diets is their different
amounts of most vitamins and E.F.A. used for the preparation of
premixtures I, II and III. Diet J contains premix I. As a source of
E.F.A. a commercial product with 51% linoleic (18:3 w6) and 17% linolenic
(18:2 w6) acid was used (Table 1, I). All diets were approx-
imately isocaloric. The caloric values used for the calculations were
9.0 calories per gram of fat, 3.9 per gram of protein and 1.6 per gram
carbohydrate (P H I L L I P S et al., 1948, PHILLIPS and HOCKWAY, 1959,
The duration of the experiment was considered quite sufficient
because, on the basis of already published papers dealing with rain-
bow trout and other salmonoid diets, this length of time appears to be
adequate for studies of vitamin deficiency, effects of essential fatty
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### Table I. Chemical composition and calculated calorie levels of the tested diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Animal</th>
<th>Plant prod.</th>
<th>Premix</th>
<th>Prem. animal</th>
<th>Plant</th>
<th>Total</th>
<th>Fat</th>
<th>Fiber</th>
<th>N-free extract</th>
<th>Ash</th>
<th>Water Cal/Kg</th>
<th>Calorie protein ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>66</td>
<td>32.6</td>
<td>1</td>
<td>1.4</td>
<td>43.95</td>
<td>12.03</td>
<td>35</td>
<td>7.5</td>
<td>2</td>
<td>11.1</td>
<td>13.7</td>
<td>9</td>
</tr>
<tr>
<td>H</td>
<td>66</td>
<td>31.5</td>
<td>1</td>
<td>2.4</td>
<td>43.95</td>
<td>11.93</td>
<td>35</td>
<td>8.6</td>
<td>2.1</td>
<td>11.2</td>
<td>13.2</td>
<td>9</td>
</tr>
<tr>
<td>I</td>
<td>66</td>
<td>30.44</td>
<td>1</td>
<td>3.16</td>
<td>43.95</td>
<td>11.43</td>
<td>35</td>
<td>8.6</td>
<td>2.1</td>
<td>11.2</td>
<td>13.2</td>
<td>8.9</td>
</tr>
<tr>
<td>J</td>
<td>66</td>
<td>32.6</td>
<td>1</td>
<td>1.4</td>
<td>43.95</td>
<td>12.14</td>
<td>56</td>
<td>8.2</td>
<td>2.9</td>
<td>11.7</td>
<td>12.2</td>
<td>8.9</td>
</tr>
</tbody>
</table>

### Table III. Growth characteristics and mortalities during the experimental time.

Initial average weight per fish: 0.25 g.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td><strong>Diet</strong></td>
<td><strong>G</strong></td>
<td><strong>H</strong></td>
<td><strong>I</strong></td>
</tr>
<tr>
<td>Mean value of fish (g)</td>
<td>1.76</td>
<td>1.69</td>
<td>1.58</td>
<td>1.73</td>
</tr>
<tr>
<td>Feed conversion factor</td>
<td>0.9</td>
<td>1.14</td>
<td>1.29</td>
<td>1.0</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.98</td>
<td>1.6</td>
<td>1.39</td>
<td>1.77</td>
</tr>
<tr>
<td>Mortality %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin/Mineral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>Thiamine</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>75.4</td>
<td>15.4</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Cobalamin</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>150</td>
<td>150</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Neuronic acid</td>
<td>50</td>
<td>75</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>A/As**</td>
<td>30</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Menadione</td>
<td>1.24</td>
<td>2.9</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Tocopherol**</td>
<td>60</td>
<td>30</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Pantothenc acid</td>
<td>50</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mea-Inositol</td>
<td>20</td>
<td>200</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>p-aminobenzoic acid</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>500</td>
<td>350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trace minerals***</td>
<td>100</td>
<td>100</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Essential fatty acid</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* Vitamin and trace minerals in mg/kg of diet. Essential fatty acids in g/kg of diet.
** Vitamins in IU/g A, 500.000, D₃, 100.000, E, 500.
*** Percentage composition: Mn, 12; Fe, 4; Cu, 0.4; I, 0.25; Zn, 0.12; Co, 0.4.


Protein efficiency ratio was defined as the gain in weight per gram of protein eaten and food conversion factor was defined as the gained weight per gram of dry feed consumed. Some of the obtained data were subjected to analysis of variance.

3. RESULTS

Mean values of fish weight (g), food conversion factors and protein efficiency ratios were not significantly different between fishes fed on the tested diets throughout the experimental time (Table III).
<table>
<thead>
<tr>
<th>Diet</th>
<th>Average final wt (g)</th>
<th>% wt gain</th>
<th>Food conversion Factor</th>
<th>Protein efficiency ratio</th>
<th>Hematocrit, Accord. %</th>
<th>% carcass glycogen</th>
<th>Liver %</th>
<th>% dry mat.</th>
<th>Moist. %</th>
<th>Prot. %</th>
<th>Fat. %</th>
<th>Ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>5.54</td>
<td>615.6</td>
<td>1.32</td>
<td>1.22</td>
<td>27.7±0.7</td>
<td>0.77</td>
<td>21.9±3.2</td>
<td>4.3±0.2</td>
<td>76.2</td>
<td>73.67</td>
<td>12.27</td>
<td>11.65</td>
</tr>
<tr>
<td>H</td>
<td>5.45</td>
<td>605.6</td>
<td>1.41</td>
<td>1.19</td>
<td>28.3±0.3</td>
<td>1.54</td>
<td>23.5±2.3</td>
<td>3.8±0.3</td>
<td>78.1</td>
<td>76.2</td>
<td>13.3</td>
<td>11.36</td>
</tr>
<tr>
<td>I</td>
<td>5.16</td>
<td>573.3</td>
<td>1.31</td>
<td>1.12</td>
<td>28.0±0.0</td>
<td>0.77</td>
<td>23.9±2.2</td>
<td>4.6±0.4</td>
<td>77.6</td>
<td>73.4</td>
<td>13.6</td>
<td>11.6</td>
</tr>
<tr>
<td>J</td>
<td>5.34</td>
<td>593.3</td>
<td>1.43</td>
<td>1.16</td>
<td>28.0±0.8</td>
<td>1.54</td>
<td>48.4±2.9</td>
<td>3.0±0.2</td>
<td>78.1</td>
<td>74.29</td>
<td>11.84</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*Means values ± SE

**mg of glycogen in g of liver
At the end of the experiment the accumulated mortality was higher in the groups of fish on diets H and J, while no statistical difference (P < 0.05) was observed in hormone values between the experimental groups. The liver analyses showed a great statistical difference (P < 0.05) in glycogen levels, the highest percentage content being observed in fish which received diet J. (Table IV). Also, the analyses of livers of fish for total fat content showed differences between all the investigated diets (P < 0.05), mainly between fish which received diets I and J. No significant difference was observed between all groups in total food conversion factors and total protein efficiency ratios. The highest carcass protein content was observed in fish on diet H, the other protein levels being quite close to each other. Fish which received diet I showed the lowest carcass fat and ash percentages, while fat and ash contents appeared quite similar in the other groups. Finally, only a slight difference showed the carcass moisture determinations between all groups (Table IV).

During the experimental time no signs of any vitamin, E.F.A, and trace elements deficiency was observed, nor any gross pathological change in any of the fish, which throughout the period of the experiment showed normal appetite and coloration.

4. DISCUSSION

Generally, the growth rate of all groups of rainbow trout fry used in this experiment is quite sufficient, taking into consideration the existing experimental conditions and data from related works.

The amount of the source of E.F.A. added to G diet seems to be adequate, since this diet gave the highest final average fish weight and since this fish population show normal levels of liver fat and glycogen percentages, as well as carcass protein, fat, ash and moisture levels. On the contrary, even though diets H and I contained ten times more the amount of the source of the E.F.A. than diets G and J, final average fish weight in the groups fed H and I were very close, even lower to those of the groups fed diets G and J. So, it seems likely that the present results indicate a significant improvement of growth rate and reduction of mortality when 3 and 6 acids are added to dry diets of salmonids, even in very low amounts, as previously have been reported (Liu et al., 1967, Nicollid and Woodside 1962, Yu and Sinnhuber, 1972, 1972).

The presence of higher amounts, of vitamin E, ricinocetic acid, vitamin K2 and inositol in premix H and III than in premix I, did not show any remarkable difference in the growth characteristics of the tested fishes, except in the case of the observed highest carcass protein

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level, of fish fed on diet H, which could be due to the vitamin combination of premix H. Similarly, no differences were observed between fish on received diet G and H—without additional choline—with those which received diets H and T—with additional choline—using for their evaluation the growth rate, mortalities and liver fat content. Taking, also into consideration the fact that when choline is mixed with other vitamins, it reacts with them and with many chemical compounds and that, probably, this mixing causes a deleterious effect on the vitamin mixture (FOUGER et al., 1964), it must be pursued to supply dry diets with the proposed amount of choline by using in the formulation of rations, ingredients with considerable level of this vitamin (FOUGER and BANK, 1967).

Fish fed on diet J show the highest liver glycogen content which may be due to the replacement of dry delarted silk-worm pupae with other animal-origin ingredient. Table IV shows that this high liver glycogen percentage is accompanied by the lowest liver fat content and the excess fat level. Also, the chemical composition of diet J (Table I) indicates that it is almost identical with the rest of the tested diets. These findings support the conclusion that this high liver glycogen level, probably, occurred, because of a disturbance of the amino acid balance, which may have existed in this diet, caused by replacement of silk-worm pupae (TOUDET et al., 1967). However, this is something which remains astonishing, since the growth rate of the fish fed on this diet is quite close to those fed on diet G. Special care was also paid to avoid, from the formulation of the diets, ingredients which naturally contain factors, as cyclopropanoid fatty acids, which have been reported to disturb the normal biochemical pathways of fish livers, by accumulation of high glycogen levels (MALENKA et al., 1974). Also, the fact, that diet G, supplemented with the lowest amounts of the majority of the vitamins used and E.F.A., gave good growth characteristics, show that this diet contained the sufficient supplementation of vitamins and E.F.A. premix.

The present results suggest that when dry trout diets are produced, the supplementation of the amounts of vitamins and E.F.A. could be adjusted, to a considerable degree, according to their naturally contributed amounts by the used ingredients.

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REFERENCES


