

UNIVERSIDADE DO ALGARVE
Unidade de Ciências e Tecnologia dos Recursos Aquáticos

**X-RADIATION FOOD INTAKE STUDY
ON RAINBOW TROUT (*Oncorhynchus mykiss*)
FED DIFFERENT PELLET SIZES RATION**

Submitted in completion of MSc in Aquaculture

by:

Manuel da Silva Costa

FARO

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(*Oncorhynchus mykiss*) - Costa,

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ABSTRACT

In aquaculture there is not enough scientific studies of feeding standards, and the improvement and implementation of this knowledge could save labour and money for fish farmers and for the feed industry.

The objective of the study was the evaluation of appetite of several sizes of rainbow trout (*Oncorhynchus mykiss*), reared in intensive cultivation, for different extruded pellet diameters.

Subsequent trials were carried out, based on the simultaneous observation of data, which was possible, due to the versatility of X-ray method described by Talbot & Higgins, (1982), which become more conclusive the results.

That only one pellet size was enough to feed the trout, weighing between 25g and 1 000g, was the most relevant conclusion from trial.

RESUMO

Em aquacultura não existem suficientes estudos científicos sobre nutrição, apesar de a eficiência neste campo conduzir à redução nos custos de produção, quer dos piscicultores como das fábricas de ração.

Como objectivo, esta experiência propôs-se a avaliação do apetite de truta arco-íris (*Oncorhynchus mykiss*), em cultura intensiva, de vários tamanhos, em relação com granulado extrudado de diferentes secções.

A experiência inicial foi complementada com base na observação simultânea dos dados, só possível devido à versatilidade do método de raios X descrito por Talbot & Higgins, (1982), o que tornou mais conclusivos os resultados alcançados.

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1. INTRODUCTION

Rainbow trout, a salmonid classified in the genus *Oncorhynchus*, as a single species *O. mykiss*, consists of populations with diverse biological characteristics, such as inland and coastal. The natural geographic distribution follows the coastal northern Pacific from Mexico - U.S.A. border, up to Canada and the Russian peninsula of Kamchatka.

In the natural environment, trout when fry, consume plankton, but as they increase in size, there is a shift in food pattern to insects and crustaceans, and thence to fish (Cho *et al.*, 1991).

The hatchery propagation of this most widespread salmonid species, was probably first carried out in the early 1870's in California (Behnke, 1979).

In intensive aquaculture salmonids are usually fed with dry extruded pellets.

The biometric relationship between body size and food size, is considered relevant to understand the limits of size of the particles that fish of different sizes are able to ingest. It is necessary to document, for each species and dimension of fish used in aquaculture, the range of food particle sizes capable of being ingested and those giving optimal growth (Wallace *et al.*, 1989).

However, at present there is not enough knowledge about the best pellet sizes for an optimisation of fish production (Cho, 1992).

It will be very useful to understand the relation between fish sizes and food dimensions with the objective of improving food intakes, conversion rates and reduction of food waste. The savings in labour and money for fish farmers and for the feed industry could be very relevant, as well as the obvious reduction of pollution of the environment (Koskela *et al.* 1991).

The appetite is directly connected to growth of fish and consequently to economic improvement of aquaculture. A maximum growth and food

efficiency occurs when fish are fed to appetite - this is a general characteristic of living creatures (Talbot, 1994).

The study of interactions between feeding biology and feeding regimes is crucial for the success of fish farming. The appetite is under multifactorial control involving metabolic, neurophysiological and hormonal mechanisms. In fish farming, some relevant factors influencing fish appetite are food dimensions, feeding frequency, water temperature and behaviour (Fänge and Grove, 1979; Fletcher, 1984; Jobling, 1986; Smith, 1989).

1.1. FOOD DIMENSIONS

Food size, is usually the strongest stimulus eliciting prey capture in adult fishes (Kislalioglu and Gibson, 1976).

The shape of food influenced food captures, in juvenile Atlantic salmon, *Salmo salar* L., and long pellets were preferred to round ones (Strademeyer, 1989).

Werner and Hall (1974), present evidence, based on results from data on bluegill sunfish (*Lepomis macrochirus*), that the size selection of prey by fish is based on optimal foraging, by making a model relating search and handling time to energy return. At low absolute abundance, prey of different size are eaten as encountered. In accordance with this theory, as prey abundance is increased, size classes are dropped sequentially from the diet.

While setting the absolute upper limit for food size at ingestion, is not a good indicator of the size of food particles actually consumed by fish. Experimental data show that few, if any, fish species constantly choose food particles of the maximum size ingestible (Wallace *et al.*, 1989).

1.2. FEEDING FREQUENCY

Frequent feeding appears to be advantageous for very young fish (Shelbourne *et al.*, 1973) or in fish populations held at very high density (Holm *et al.*, 1990).

Grayton and Beamish (1977) fed 15g rainbow trout, held at 10°C., at frequencies ranging from one meal every second day to six meals per day. No significant differences in daily food intake, growth rate, or body composition was found at feeding frequencies of two or more meals per day. Cho (1992) feeding rainbow trout for 6 days per week found no effect on growth rate compared to feeding every day, but feeding for only 5 days per week resulted in significant growth reduction.

Generally, the ration consumed per meal is proportional to the degree of stomach emptiness and as evacuation rate increases with increasing temperature optimum feeding frequency may be temperature dependent (Smith, 1989). Brown trout (*Salmo trutta*) voluntarily consumed one meal per day at 4°C., and 3 meals per day at 18°C. (Elliott, 1975).

Storebakken and Austreng (1988) concluded that rainbow trout eat continuously when food supply is limited, but they develop a "meal time" behaviour when fed excess rations.

Trout fed appropriately sized pelleted diets appear to ingest sufficient for satiation in one hour or less and the majority of the ration is consumed within 15 minutes. Grove *et al.* (1978) determined the satiation time for rainbow trout consuming a maximal ration as:

$$S = 0.031 W + 0.868 T + 29.15.$$

Where S is satiation time (minutes), W is body weight (grams) and T is temperature (°C.).

In juvenile sockeye salmon (*Oncorhynchus nerka*) fed a single satiation ration per day at 15°C., maximum appetite occurred after 11 hours, when 10% (approximately) of the previous meal remained in the stomach (Brett, 1971).

For rainbow trout weighing up to 300g, maximum food intake (F.I., in grams) resulting from a single satiation meal, varies with body weight (W, in grams) according to $F.I. = 0.024 W^{1.1}$, while stomach volume (V, in millilitres) was also found to vary with body weight, $V = 0.075 W - 0.8$ (Grove *et al.*, 1978). The stomach capacity and the degree to which the stomach was filled before feeding was terminated may be important parameters when considering feeding regimes.

1.3. TEMPERATURE

Rainbow trout live normally within a temperature range of approximately 1°C. to 20°C. A general optimum for growth rate and food conversion rate, is 15°C. approximately (Cho *et al.*, 1991).

Daily ration varies with body weight and water temperature and tables are available which give some approximation to the required ration. Current practices for the cultivation of salmonids employ feeding rates of around 2% to 3% of body weight per day. However, according to Talbot (1994), the appetite of fish at each feeding is not predictable. The key to maximising growth and minimising food waste is always to feed to appetite on a meal-to-meal basis.

1.4. BEHAVIOURAL CONSIDERATIONS

Behavioural studies of salmonids have shown that generally, dominant fish gain preferential access to food consumption and have higher growth rates than subordinate fish (Huntingford and Thorp, 1992).

Food supply limited in quantity, space or time leads to undesirable high levels of competition (Noakes and Grant, 1992).

Trout, like other animals, are influenced by biorhythms. Fish fed in the morning have a protein metabolism more active as compared with those fed in the afternoon. However, fishes fed in the afternoon have richer fat deposits (Boccignone *et al*, 1991).

Salmon parr, fed *ad lib.* at different times of the day show particular feeding rhythms:

- Midday, 90% feeders; Dusk and Dawn, 70% and Midnight only 7% (Talbot and Higgins, 1982).

However, salmonids, like other farmed animals, seem able to adapt physiologically and behaviourally to a wide range of feeding patterns entrained by food availability (Talbot, 1994).

1.5. OBJECTIVE

The objective of the study design was the evaluation of appetite of several sizes of rainbow trout, reared in intensive cultivation, for different pellet diameters.

The eventual biological constraints of trout, in relation to food pellet sizes were studied and the biometric relationships between fish body and mouth dimensions were analysed.

2. MATERIAL AND METHODS

The methodology chosen to analyse the fish appetite for different feed dimensions intake was X-radiation, a direct measurement method of feed intake, described by Talbot & Higgins, (1982).

This technique for measuring fish food intakes can be used for different studies, such as appetite, meal size and frequency, rate of gastric evacuation or evaluation of inter and intra animal variability in food intake and metabolic efficiency which may lead to a greater understanding in growth efficiency, with application for genetic selection and breeding programs (McCarthy *et al*, 1991).

The method has advantages compared to others more stressful and unnatural for fish, such as examination of gut contents. Large animal population may be studied at moderate cost, in comparison with other methods, for example direct observation of feeding activity (Tytler & Calow, 1985).

Additionally, trophic dynamics studies in fish normally considered too small for investigations, can be practised successfully using this method (Talbot & Higgins, 1982).

Compared to radioisotope methods, X-radiation studies do not involve the handling of radioactive substances.

Another positive characteristic of this method and relevant for the experiment is the fact that the feed intakes are voluntary for fish, which reduces possible negative effects of experimental design, specially concerning stress in stock. The rapidity of X-ray plate analysis makes this method very flexible and adaptable during the trial procedures.

2.1. ENVIRONMENTAL CONDITIONS

The experiment was carried out during 31 days, between August 17th and September 16th 1995 at Lerang Research Centre, near Stavanger, Norway.

Rainbow trout were maintained in an indoor glassfibre tank 1.0m high * 2.0m wide * 2.0m deep, filled to a depth of 0.7m, giving in a water volume of 2.8m³.

The tank was supplied with fresh water from a nearby lake, at ambient temperature. It was also provided with oxygenation.

During the experiment, water temperature in the tank fell from 18.1°C. initially to 11.5°C. at the last observation, and dissolved oxygen concentrations varied from 7.5mg/l (76.9%) to 12.7mg/l (116.2%). The saturation oxygen in water (%), were registered for each temperature and are shown in **TABLE I**.

TABLE I - The temperature (°C.), dissolved oxygen data (mg/l) and saturation (O₂, %), observed during six sampling dates.

Sampling Dates	Temperature (°C.)	Dissolved O ₂ (mg/l)	Saturation O ₂ (%)
95/8/24	18.1	9.2	97.6
95/8/28	16.5	7.5	76.9
95/9/01	15.8	7.6	76.9
95/9/05	16.0	7.6	77.2
95/9/12	15.7	8.7	87.7
95/9/16	11.5	12.7	116.2

2.2. LIVESTOCK

The rainbow trout used were from Lerang Research Centre and NLA - Kyrksæter - Øra Station.

Four groups of 150 fish were chosen, based on individual weights. The weight ranges were from 25-50g, 51-100g, 101-200g and 201-1000g. The last group was subsequently subdivided into 2 groups of fish within the weight ranges 201-500g and 501-1000g.

The total population of 600 fish, divided into 5 weight groups, weighed 106.0kg. After the first day, 3 fish weighing 0.3kg died, reducing the initial weight to 105.7kg.

The fish were acclimatised under the tank conditions described previously, before being sampled for the first time.

The average daily stock growth was estimated as 1.5% per day, taking into account the weight of fish and water temperature.

The evolution of fish weight during the trial was calculated as follows using the formula:

Final weight = (1 + growth rate)^{n.days} * Initial weight.

$$= (1.015)^7 * (105.7)\text{kg} = 117.4\text{kg}.$$

The total stock weight at first sampling (after 7 days of acclimatisation period) was calculated as 117.4kg, and stocking density 41.9kg/m³ (117.4kg/2.8m³).

It was necessary to add fish to the smallest size group, to compensate this group for the continuous “loss”, through growth, to the next size class.

In order to maintain stocking density as constant as possible, bigger fish were removed after each sampling as required.

The population parameters during the trial dates were: fish weight (kilograms), stocking density (kg/m³) and numbers of fish after mortalities, removals and additions, as show in TABLE II.

TABLE II - Fish weight (kg), stocking density (kg/m³), and numbers of fish - after mortalities, removals and additions.
Data was collected before acclimatisation periods and before and after each sampling.

Acclimatisation Period (95/8/17 - 95/8/24)	Fish Weight (kg)	Stocking (kg/m ³)	No. of Fish			
95/8/17	105,7	37,8	597			
Trial with "choice"		Before Sampling		After Sampling		
Sampling Dates	Fish weight (kg)	Stocking (kg/m ³)	No. of Fish	Fish weight (kg)	Stocking (kg/m ³)	No. of Fish
95/8/24	117,4	41,9	597	110,5	39,5	585
95/8/28	117,2	41,9	585	111,3	39,8	655
95/9/01	118,1	42,2	655	109,8	39,2	642
95/9/05	116,5	41,6	642			
Acclimatisation Period (95/9/5 - 95/9/12)	Fish Weight (kg)	Stocking (kg/m ³)	No. of Fish			
95/9/05	107	38,2	705			
Trial with "no choice"		Before Sampling		After Sampling		
Sampling Dates	Fish Weight (kg)	Stocking (kg/m ³)	No. of Fish	Fish Weight (kg)	Stocking (kg/m ³)	No. of Fish
95/9/12	118,9	42,5	705	112,4	40,1	696
95/9/16	117,6	42	696			

The average stock density during the experimental period was 42.0 (± 0.3066) kg/m³.

2.3. FOOD

2.3.1. FOOD COMPOSITION

The feed used was in the form of extruded pellets, produced by Skretting^a /_s, Stavanger. Four pellet sizes were used and references are shown in TABLE III.

TABLE III - Pellet dimensions and commercial names.

Pellet Ø	Commercial Name
2.5 mm	"6+6 Sjøvann"
3.0 mm	"Royal Redline"
4.0 mm	"Royal Redline"
6.0 mm	"Royal Redline"

This feed composition was based in the following raw materials: fish meal, fish meal “low temperature”, fish oil, carbohydrates, fish protein concentrate, shrimp meal (in 2.5mm pellets), vitamins and minerals.

The composition and dietary energy of the feed supplied by the manufacturer, are shown in TABLE IV.

TABLE IV - Chemical composition and dietary energy of the pellets.

Pellet Ømm	Chemical Analysis						Energetic Values			
	Astax. mg/kg	Protein %	Fat %	Carboh. %	Ash %	Moist. %	Dig. Energy MJ/kg	Protein %	Fat %	Carboh. %
2.5	-	47	25	11	9.0	10.0	20.8	49	44	7
3.0	60	47	26	12	9.0	6.0	21.2	48	45	7
4.0	75	47	28	10	9.0	6.0	21.7	46	48	6
6.0	75	46	30	9	8.5	6.5	22.2	45	50	5

Astax. - Astaxanthin (pigment).

Carboh. - Carbohydrates.

Dig. Energy - Digestible energy.

Moist. - Moisture content.

2.3.2. RELATIONSHIP BETWEEN EACH PELLET DIAMETER AND LABEL

Pellets were labelled for subsequent detection by means of X-radiation, using Ballotini glass spheres, which were mixed into the ration during production.

Ballotini size 6 was used to label the 6mm pellets and size 8 for the remaining pellet diameters - 2.5mm, 3.0mm and 4.0mm.

The relationship between weight of food and label content was calculated, for each food pellet size, using data obtained from X-ray measurements of the numbers of Ballotini in accurately weighed samples of labelled feed.

The exact relationships between the different pellet sizes and label content, were calculated by regression analyses. All labelled feeds had a highly significant relationship between food weight and number of Ballotini ($p < 0.01$) and none of the coefficients of correlation (R^2) were lower than 0.96.

The number of pellet per Ballotini was calculated using the relationship between the number of glass spheres used per gram of food and the average weight of each pellet size, as shown in TABLE V.

TABLE V - Relationship between no. of Ballotini per food (g) and no. of different pellet diameters (mm), per Ballotini.

Pellet Ø	Equation	R ²	Pellet Weigh	No.Ballotini/g	No.Pellets/Ballotini
2.5mm	$y=0.0717x-9.399*10$	0.9831	0.026g	140.781	2.732
3.0mm	$y=0.0692x+6.969*10$	0.9924	0.037g	142.268	1.901
4.0mm	$y=0.0634x-0.0144$	0.9947	0.087g	155.820	0.737
6.0mm	$y=0.2286x-0.1854$	0.9636	0.293g	51.855	0.658

R² - Coefficients of correlation.

2.3.3. SIZE ADJUSTED FOOD INTAKE

In order to compare food intakes of rainbow trout, with different sizes, per unitary weight (kilogram) of fish, the intake values of individual trout were size-adjusted according to the allometric relationship described by the formula (Jobling, 1993): $F.I.a. = F.I. / F.W.^{0.75}$, where F.I.a. is the adjusted food intake (per kilogram of fish / day), F.I. is food intake (per kilogram of fish / day), F.W. is the weight of fish (grams) and a general exponent of 0.75 was used.

2.4. TRIAL FOOD INTAKE

The X-ray apparatus used in this study was a Todd Research 80/20 model, Alfa Strutturix D7Dw plates and protection apron.

Metacaine (tricaine, 50mg per litre of water for 4-6 minutes), was used as anaesthetic.

The food intakes of rainbow trout, were evaluated after analysis of X-ray plates of fish fed with labelled pellets.

The Ballotinis counted within the plates were related to unitary weight of food. X-ray observations took place every 4 days, at least one hour after the last meal - to avoid possible regurgitation during sampling.

It was considered important to test the preferences of fish when the size of food was free choice and also, when that was not an option.

As a consequence, the data obtained from the experiments, were subdivided according to the following complementary trials - “food intake with choice” and “food intake with no choice”, which tested the results of each other.

2.4.1. TRIAL FOOD INTAKE WITH “CHOICE”

In this trial, the constitution of fish meal corresponded to the equivalent weights of four mixed pellet sizes: 2.5mm, 3.0mm, 4.0mm and 6.0mm.

Taking into account fish size and ambient temperature, satiation ration was considered as 4% body weight per day.

Feeding was carried out twice daily, at 08⁰⁰ hour and at 14⁰⁰.

The two daily meals weighing 4kg each (near double of the expected fish appetite), were supplied in order to provide fish free choice concerning food dimensions.

Each X-ray observation included approximately 40 randomly selected fish per group size.

The adaptation period and the sampling dates for trial, are shown in TABLE VI

TABLE VI - Calendar of trial with “choice” .

1 - 2 - 3 - 4 - 5 - 6 - 7 - A - 9 - 10 - 11 - B - 13 - 14 - 15 - C - 17- 18 - 19 - D
I Acclimatisation Period I

Letters correspond to sampling dates and numbers to remaining days.

As preferences were expected to be observed, the live stock was divided into the following size ranges: 25-50g, 51-100g, 101-200g, 201-500g and 501-1000g, and could choose among 4 pellet dimensions. On each sampling date, a different labelled pellet size was tried, as shown in TABLE VII.

TABLE VII - Calendar of meal compositions with “choice”, where letters correspond to sampling days, and pellets sizes 2.5mm, 3.0mm, 4.0mm and 6.0mm were used.

A	B	C	D	Other Days
3,0mm	2,5mm	3,0mm	2,5mm	2,5mm
2,5mm	6,0mm	4,0mm	3,0mm	3,0mm
4,0mm	3,0mm	2,5mm	6,0mm	4,0mm
6,0mm	4,0mm	6,0mm	4,0mm	6,0mm

Standard and **labelled food**.

2.4.2. TRIAL FOOD INTAKE WITH “NO CHOICE”

The methodology of the previous trial was used. However, the number of pellet diameters was reduced to the 4.0mm and 6.0mm, without mixing.

The evaluation of the appetite of the same fish ranges, for the large food, when they had no choice concerning pellet sizes, was expected in this trial.

A second acclimatisation period of one week, to the new dietary characteristics was followed before the first sampling date, as shown in **TABLE VIII**.

TABLE VIII - Calendar of trial with “no choice”.

- 21 - 22 - 23 - 24 - 25 - 26 - E - 28 - 29 - 30 - F.
I Acclimatisation Period I

Letters correspond to sampling dates and numbers to remaining days.

2.5. TRIAL FEEDERS

This study estimated the percentage of rainbow trout which did not feed.

A correction factor was introduced, in order to estimate the “blind eaters” - fish which may have been eating pellets without Ballotini, considering the fact that, especially with the smaller pellets, not all of them had at least one Ballotini, as shown in **TABLE V**.

The correction factor was based on the Poisson Distribution, which may be used when the probability of one event is rare:

$$P (y = Y = 0) = (\mu^y / Y !) e^{-\mu} = e^{-\mu}.$$

The probability (P) of individual fish (Y) within a certain group (y) not fed from a certain pellet dimension was calculated using the previous formula, where, in this case, the average number of pellets per fish in each fish group (μ) and the deviation of this distribution had the same value. The Neperian logarithm (e).

Obviously, the correction factor only could increase the observed values and when (real) values were superior to the calculated ones, the former were chosen as being more correct.

2.6. TRIAL BIOMETRIC RELATIONSHIPS BETWEEN BODY AND MOUTH DIMENSIONS

Fifty five trout, chosen at random, were also weighed and measured - (fork) length, and mouth gape (width and height).

Dimensional measurements were analysed and related as follows: fork length and mouth width; body weight and mouth width; and body sections and mouth sections.

The regression formula (y) and correlation coefficient (R^2), for these relationships were determined.

Mouth section was considered as a circle and mouth width as the respective diameter. Considering that fish specific weight is nearly equal to unity (1g/cm³), the values of body - weight and volume, are similar. The normal measure of the body section in rainbow trout is obtained, when the fish volume is divided by the fork length.

2.7. STATISTICAL ANALYSIS

With the objective of evaluation of adjusted food intakes, Ballotini ingested by fish were counted in X-ray plates and data were analysed using the programs - Packages Software Unistat Statistical (Norway) and Stat Graphic (Portugal).

The Interactions between fish size groups (F.G.) and pellet diameters (P.S.), and the importance of food intake amounts (F.I.a.), in both “choice” and “no choice” trials, were analysed statistically.

The Statistical Analysis of Variance (ANOVA), Duncan Test (95%) and Interactions were performed, in order to evaluate adjusted food intakes (F.I.a.), between 5 fish weight groups (F.G.) and 4 pellet sizes (P.S.) concerning the with “choice” trial and 2 pellet diameters with “no choice”.

For statistic purposes, data was subdivided into 20 groups (5 fish groups * 4 pellet sizes), in with “choice” and 10 groups (5 fish groups * 2 pellet sizes) with “no choice”.

Adjusted food intakes were evaluated with ANOVA for the twenty data groups (5 fish groups * 4 pellet sizes) for trial with “choice” and for the ten data groups connected to two pellet dimensions (5 fish groups * 2 pellet sizes) for trial with “no choice”.

The Statistical Duncan Interval Test (95%) analysed which data groups were significantly different, concerning food-intakes (F.I.a.), in the trials with “choice” and with “no choice”.

3. RESULTS

The appetite of fish, for each pellet diameter (P.S.), were described by the adjusted food intakes (F.I.a., g/kg of fish weight) values.

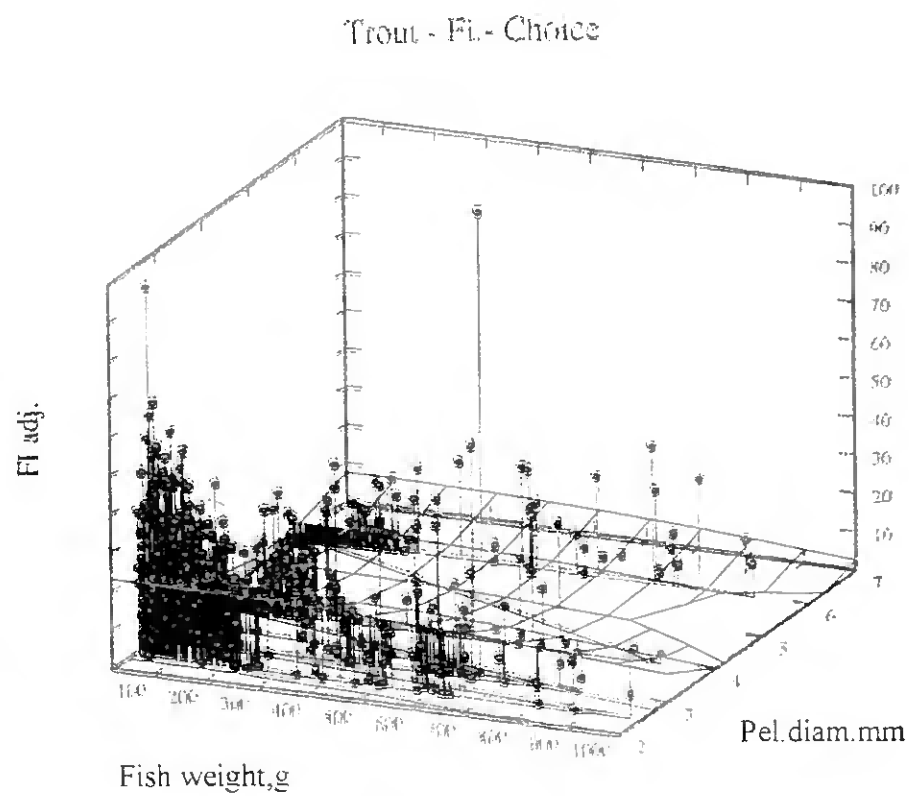
The initial experimental design consisted of a trial “food intake”. However, subsequent trials took place, based on the observation of data and, as a consequence, the results obtained from the experiments were subdivided according to the following complementary trials - “food intake with and with no choice”, “feeders with and with no choice” and “biometrics relationship between body and mouth dimensions”.

3.1. FOOD INTAKE WITH “CHOICE”

The adjusted food intakes for individual fish, are shown **FIGURE 1**.



FIGURE 1 - Individual adjusted food intakes, from trials with "choice"



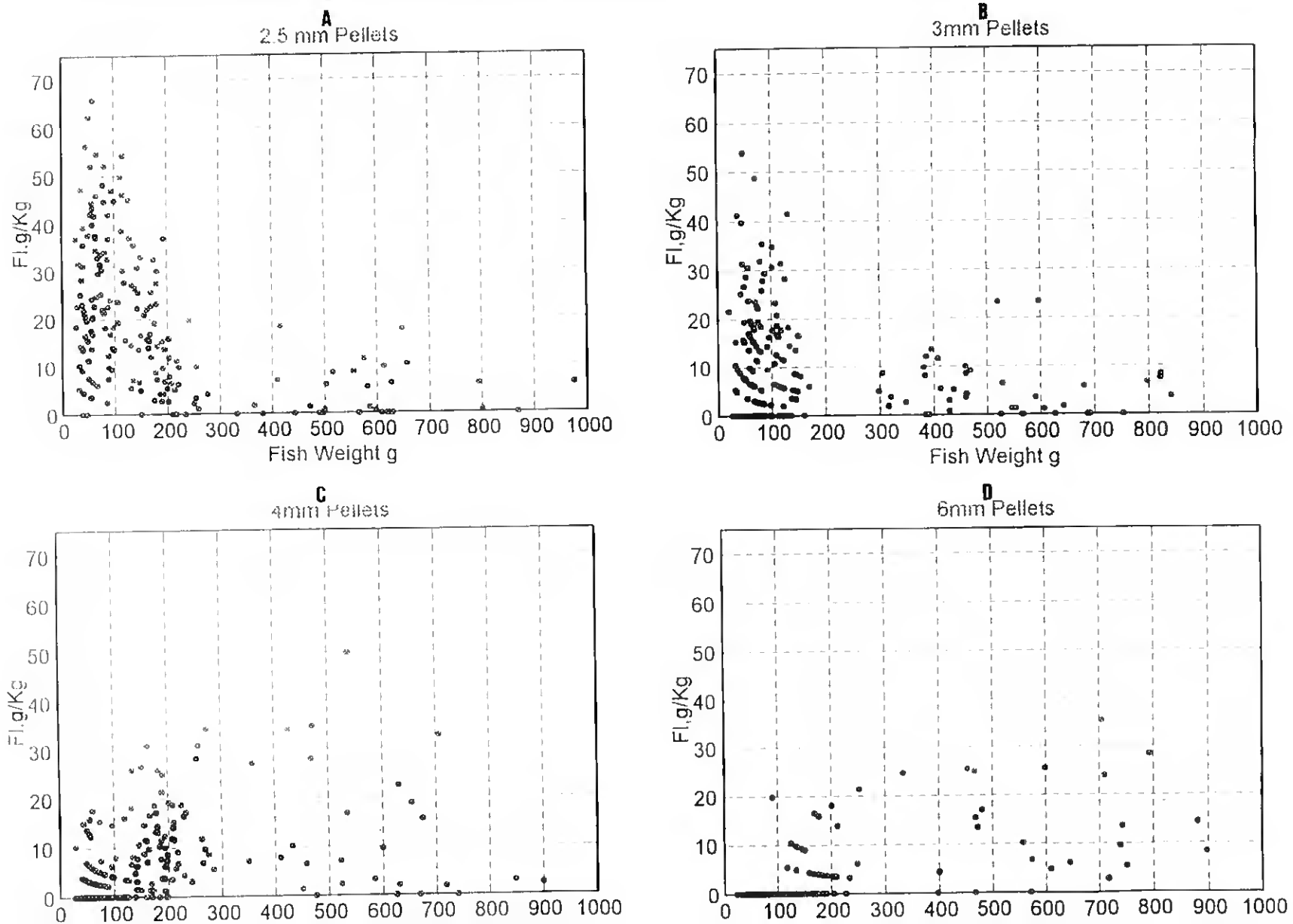
FI adj. - Adjusted food intake (g/kg of fish weight).
Pel. diam. mm - Pellet diameters (2.5, 3.0, 4.0 and 6.0mm)

The data in **FIGURE 1** was subdivided as shown in **FIGURE 2**. It represents the adjusted food intakes of each sampled fish divided for four pictures **A**, **B**, **C** and **D**, one per each pellet size.

Pictures **A** and **B** which represent adjusted food intakes for smaller pellets (2.5mm and 3.0mm), indicates a general preference of small fish to these pellet dimensions.

From pictures **C** and **D**, it appears that the bigger fish prefer larger pellets (4.0mm and 6.0mm).

FIGURE 2 - Individual adjusted food intakes, for each mixed pellet diameter (2.5, 3.0, 4.0 and 6.0mm) - trials with "choice".



Data from this trial are shown in TABLE IX, and the values represent the means of adjusted food intakes, for each 20 data groups - 5 fish groups * 4 pellet sizes.

TABLE IX - Values are means F.I.a. for 5 F.G. * 4 P.S., for trial with "choice".

F.G. * P.S.(mm)	No.fish samp.	F.I.a. (g)
1 2.5	36	22.5
1 3.0	35	11.7
1 4.0	26	2.2
1 6.0	24	0.0
2 2.5	58	28.6
2 3.0	72	10.9
2 4.0	58	3.1
2 6.0	78	0.3
3 2.5	54	20.5
3 3.0	48	10.8
3 4.0	64	7.9
3 6.0	62	1.8
4 2.5	30	5.4
4 3.0	23	5.5
4 4.0	35	14.1
4 6.0	21	13.9
5 2.5	20	5.3
5 3.0	20	4.7
5 4.0	17	11.0
5 6.0	15	13.0

F.G. - Fish weight groups 1,2,3,4,5 (25-50g; 51-100g; 101-200g; 201-500g; 501-1000g)

P.S. - Pellet diameters (2.5mm, 3.0mm, 4.0mm and 6.0mm).

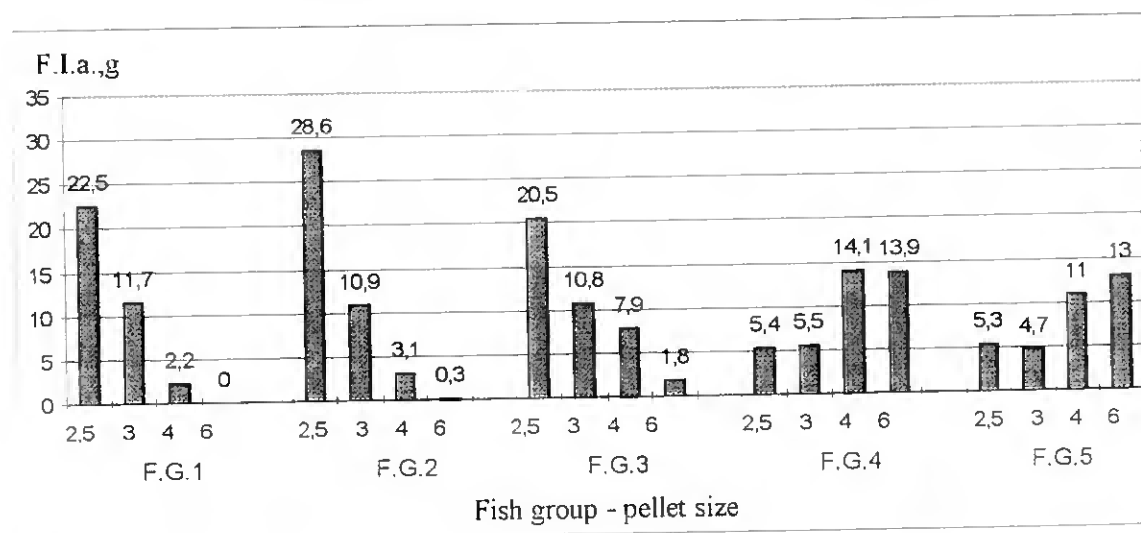
No.fish samp. - Number of fish sampled per F.G.

F.I.a. - Adjusted food intake (g/kg of fish weight).

Adjusted food intakes, as a measure of fish appetite, were divided into 20 groups of data and the rankings of the means of adjusted food intakes, for 4 pellet sizes in relation to each 5 fish groups are shown in **FIGURE 3**.

It is possible to relate adjusted food intakes within all groups of data, or only among fish groups or pellet sizes. The similarity of data from fish group 1, 2 and 3 can be seen in this figure and the preference of smaller fish for the pellet diameters 2.5mm and 3.0mm. An identical aspect can be detected between adjusted food intakes data from fish groups 4 and 5, which appeared to prefer the bigger pellets.

FIGURE 3 - Ranking of means of adjusted food intakes (F.I.a., g/kg of fish weight) for 4 pellet sizes (mm) in relation to each 5 fish weight groups (F.G.), for trial with "choice".



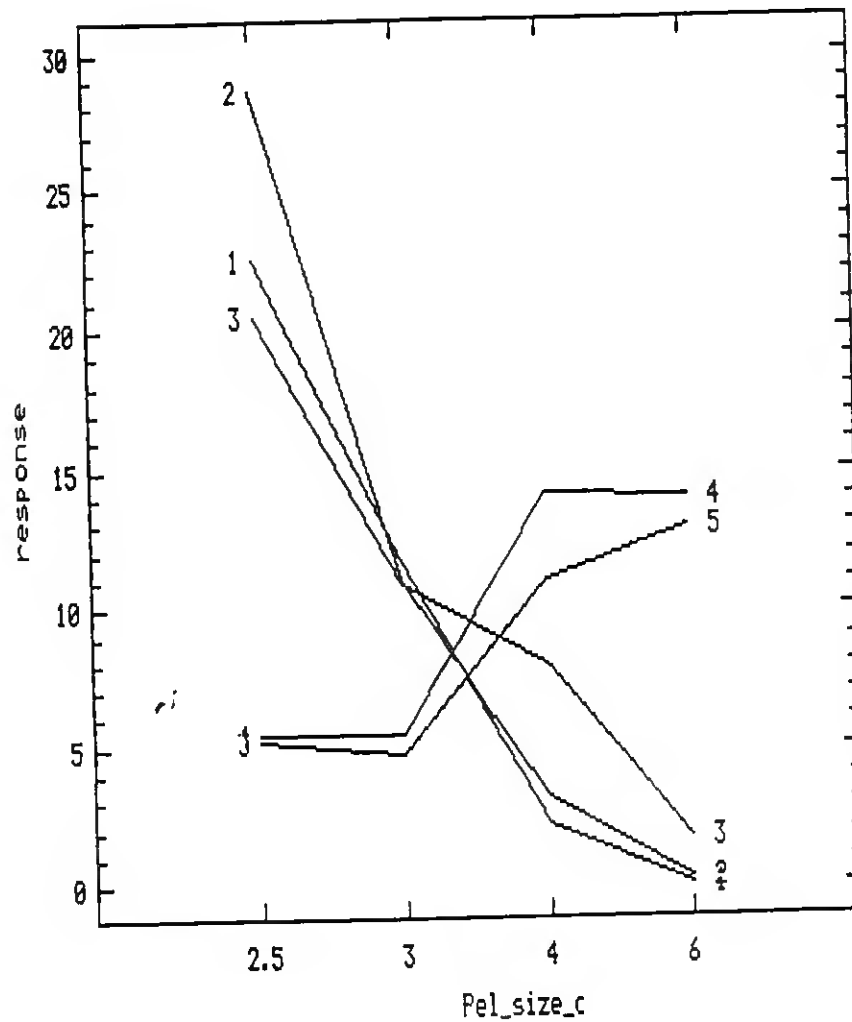
After Statistic Analyses of Variance (ANOVA), a significant difference in adjusted food intakes could be detected among the 20 groups of data (5 fish groups * 4 pellet sizes).

Duncan's Multiple Range Test (95% confidence limit) analysed, which among these 20 groups are significantly different, concerning adjusted food intakes, and associates them in 6 homogeneous ties. From the analyse of these ties, may be concluded that the smaller fish (25-200g) select the smaller pellets (2.5mm and 3.0mm). The opposite may be observed among the bigger fish (201-1000g), which preferred the large pellets (4.0mm and 6.0mm).

Statistical Interactions between the two parameters fish groups and pellet sizes, and the importance of these factors for adjusted food intakes quantities, were analysed and are shown in **FIGURE 4**, where it was observed any interactions among fish groups 1, 2 and 3 by one side and fish groups 4 and 5 on the other side.

From the analyses of this picture, two opposite feeding behaviours could be observed - 1, 2 and 3 fish groups on one side and 4 and 5 fish groups on the other side.

FIGURE 4 - Statistical Interactions between the two factors of 5 fish size groups and 4 pellet diameters, in relation to adjusted food intake for trial with "choice".



1; 2; 3; 4; 5 - Fish size groups (25-50g; 51-100g; 101-200g; 201-500g; 501-1000g).
 Pel. size c. 2.5, 3.0, 4.0 and 6.0 - Pellet diameters for trial with "choice" (mm).

3.2. FOOD INTAKE WITH “NO CHOICE”

This study analysed the relationship among the adjusted food intakes (F.I.a., g/kg of fish weight) for 200 trout weighing between 25g and 1 000g to 2 unmixed pellet diameters (P.S.) - 4.0mm, and 6.0mm.

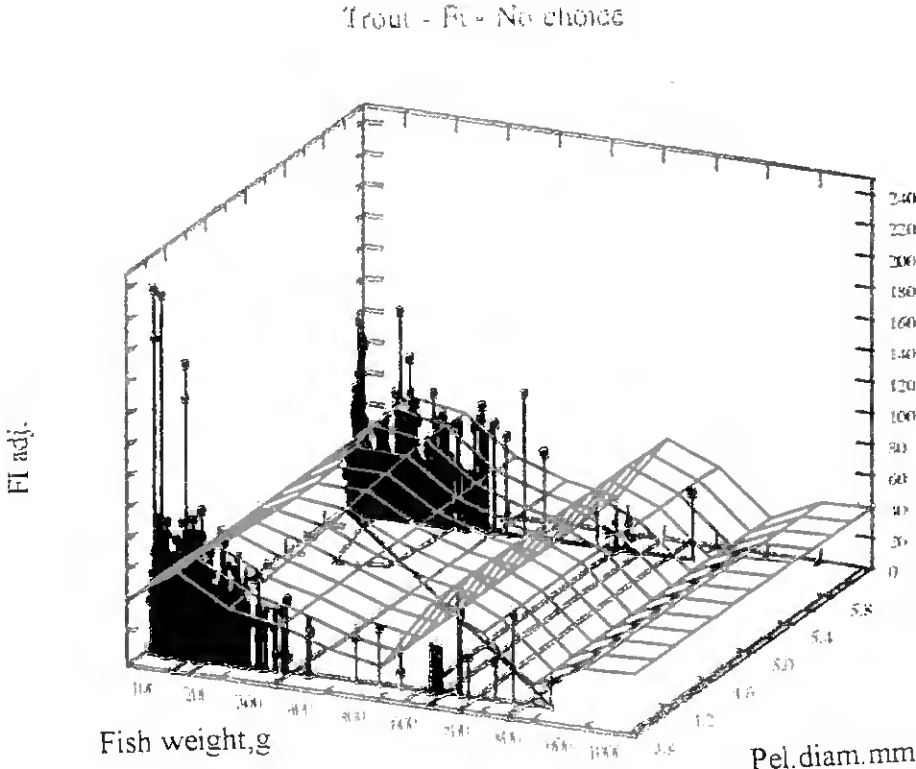
This option took place as consequence of a preliminary analyses of X-ray plates from previous trial, where the insignificant adjusted food intakes of smaller fish, in relation to large pellet sizes was obvious. After this observation, it was considered important to test the ability of smaller fish to swallow the bigger pellets, when without alternative.

The data of adjusted food intakes, for individual fish, are show in **FIGURES 5 and 6**.

The following **TABLE X** and **FIGURES 7 and 8**, display the same data, dividing into five fish size groups (F.G.) and relating them to two pellet diameters (P.S.).

Adjusted food intakes concerning each sampled fish, are shown in **FIGURE 5**, where data was related to only 4.0mm and 6.0mm pellet sizes, without mixing.

FIGURE 5 - Individual adjusted food intakes, from trials with "no choice"

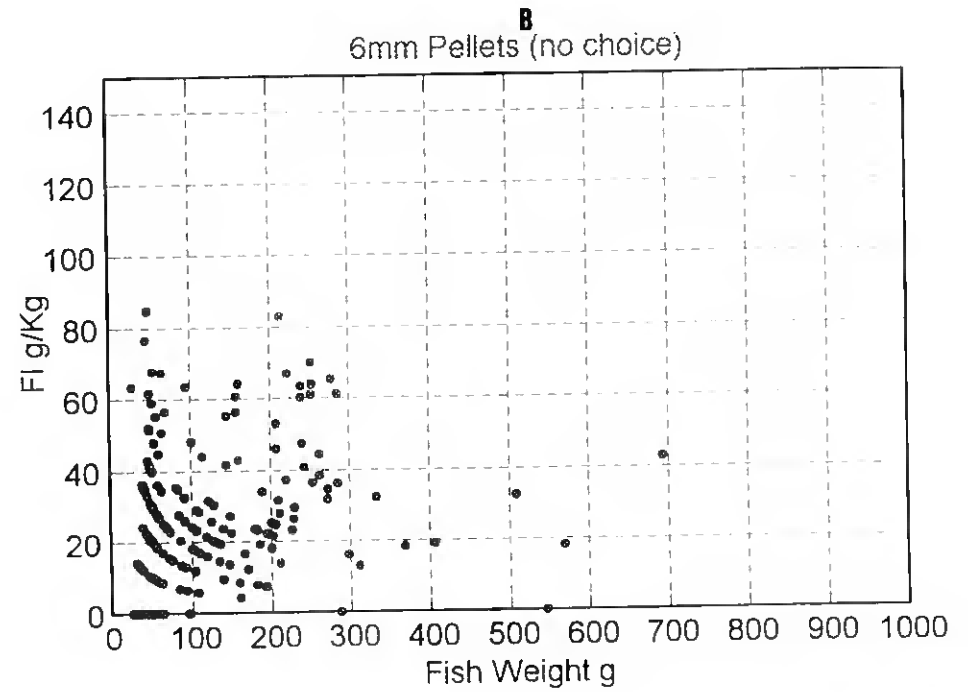
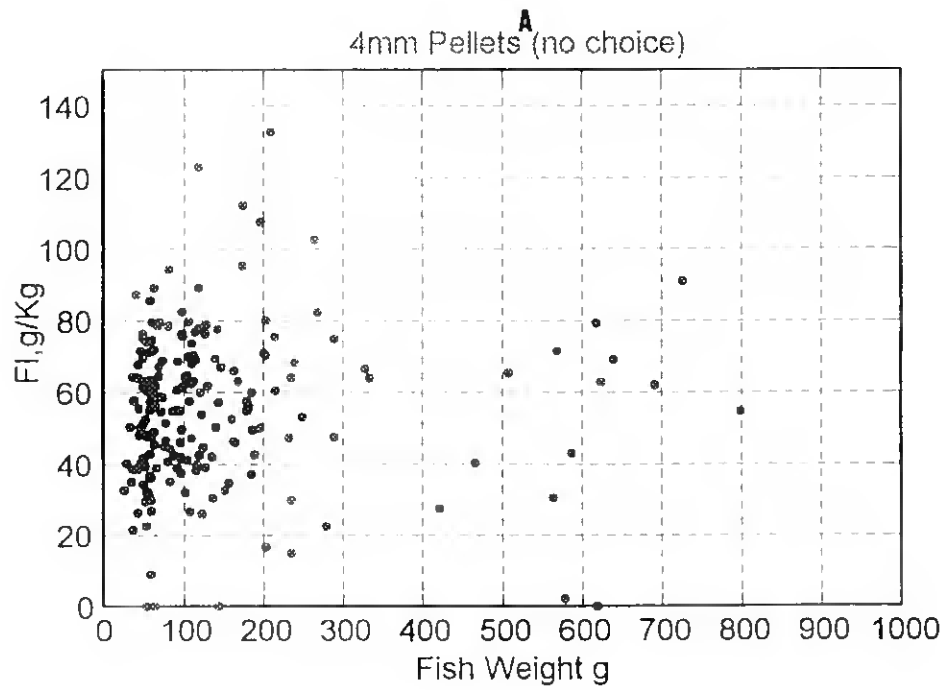


FI adj. - Adjusted food intake (g/kg of fish weight).
Pel. diam. mm - Pellet diameters (4.0mm and 6.0mm).

The data of **FIGURE 5** was subdivided as shown in **FIGURE 6**. It represent the adjusted food intakes, concerning each sampled fish and divided for two pictures **A** and **B**, one per each unmixed pellet size - 4.0mm and 6.0mm diameters.

Although results from pictures **A** and **B** look very similar, there is a general tendency for a homogeneous adjusted food intakes among total fish population and it can be observed, a bigger intake from 4.0mm pellets.

FIGURE 6 - Individual adjusted food intakes, for each pellet diameter (4.0mm and 6.0mm) - trials with "no choice".



FI, g/kg - Adjusted food intake (g/kg of fish weight).

The values obtained, represent adjusted food intakes when 5 fish size groups (similar to previous trial) had “no choice”, concerning pellet sizes. The amounts and how data was organised in this trial, are shown in **TABLE X**, which values represent the means of adjusted food intakes, for each 10 data groups - 5 fish groups * 2 pellet sizes.

TABLE X - Values are means of F.I.a., for 5 F.G. * 2 P.S., for trial with “no choice”.

F.G. *P.S.(mm)	No.fish samp.	F.I.a. (g)
1 4	32	67.3
1 6	47	25.1
2 4	76	55.8
2 6	61	23.9
3 4	57	58.2
3 6	50	25.4
4 4	23	57.8
4 6	38	37.7
5 4	12	52.5
5 6	4	23.4

F.G. - Fish weight groups 1,2,3,4,5 (25-50g; 51-100g; 101-200g; 201-500g; 501-1000g)

P.S. - Pellet diameters (4.0mm and 6.0mm)

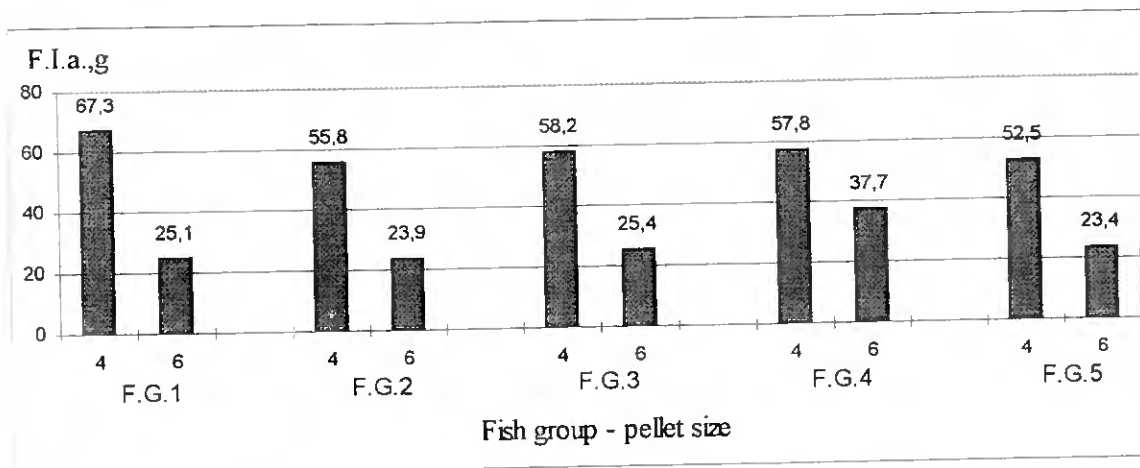
No. fish samp. - Number of fish sampled per F.G.

F.I.a. - Adjusted food intake (g/kg of fish weight).

The ranking of the means of adjusted food intakes for 2 pellet sizes in relation to each 5 fish size groups is shown in **FIGURE 7**.

It is possible to inter-relate adjusted food intakes, within all groups of data, or only among fish groups or pellet sizes, from picture, and it can be observed the similarity of data among the total fish groups and the preference of fish for the two pellet diameters.

FIGURE 7 - Ranking of means of adjusted food intakes (F.I.a.,g/kg of fish weight) for 2 pellet sizes (mm) in relation to each 5 fish size groups (F.G.), for trial with “no choice”.

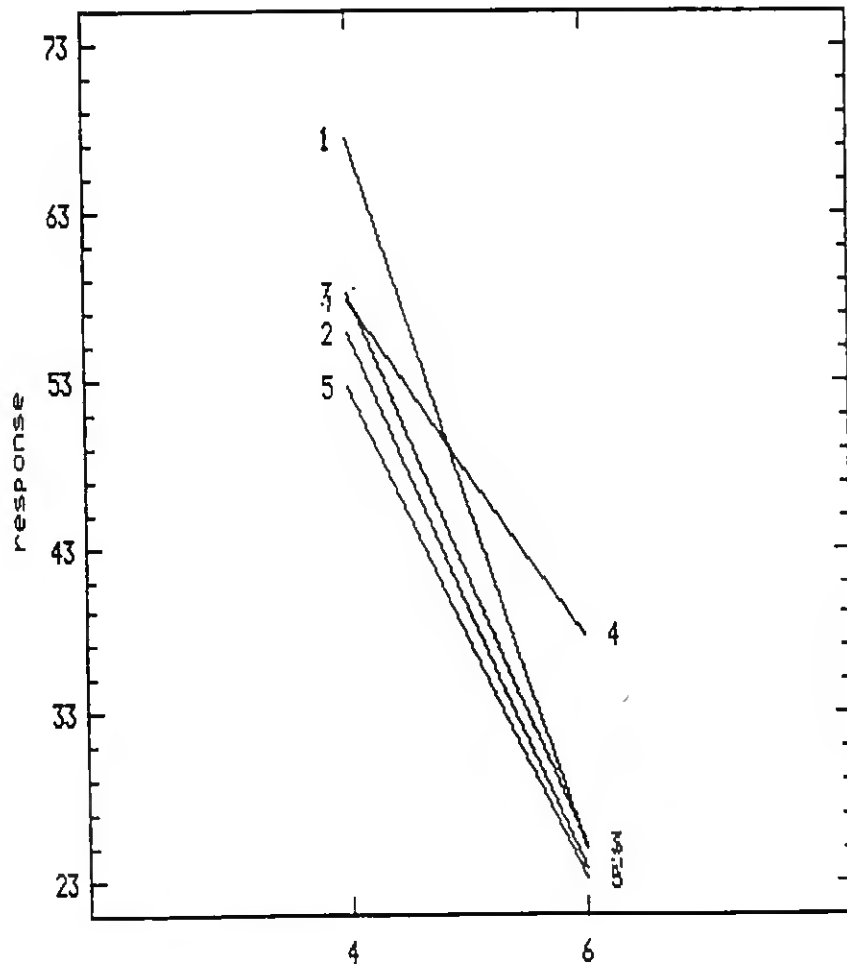


After ANOVA, it could be detected a significant difference in adjusted food intakes among the 10 groups of data. - 5 fish groups * 2 pellet sizes.

Duncan's Multiple Range Test (95% confidence limit) analysed which among these 10 groups were significantly different, concerning adjusted food intakes, and associates them in 2 homogeneous ties. From the analyse of these ties, may be concluded that when fish could not chose the size of pellets in presence (4.0mm and 6.0mm), it may be observed that the behaviour of total fish was homogeneous, concerning adjusted food intakes, and preferred 4.0mm pellets.

Statistical Interactions between the two parameters fish groups and pellet sizes and the importance of these factors for adjusted food intakes quantities is shown in **FIGURE 8**.

FIGURE 8 - Statistical Interactions between the two factors of 5 fish size groups and 2 pellet sizes (P.S.), in relation to adjusted food intake for trial with “no choice”.



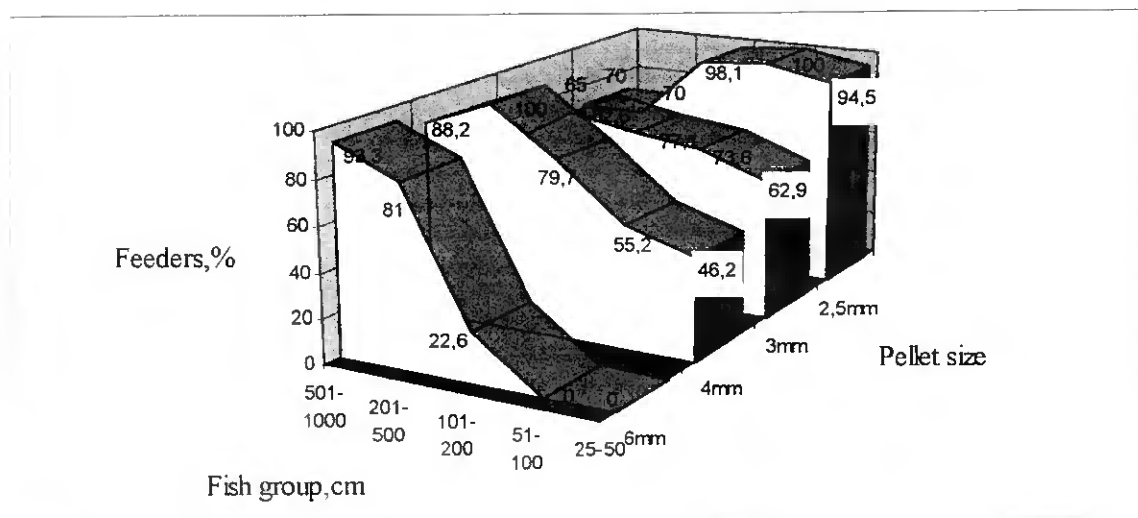
1; 2; 3; 4; 5 - Fish size groups (25-50g; 51-100g; 101-200g; 201-500g; 501-1000g).
 Pel. s. n.c. 4.0 and 6.0 - Pellet diameters with “no choice” (mm).

3.3. FEEDERS WITH “CHOICE”

The situation with food “choice” used 4 equal mixed weights of pellet sizes (2.5mm, 3.0mm, 4.0mm, and 6.0mm); and 5 F.G. (1. 25-50g, 2. 51-100g, 3. 101-200g, 4. 201-500g and 5. 501-1000g) is shown in **FIGURES 9**.

From the observation of figure, it was possible to infer that the smaller the fish was, the lower the rate of feeders, in relation to large pellets.

FIGURE 9 - Trout (Feeders %) eating at least one pellet from “choice” treatment. Fish were divided in 5 fish size groups (g) and pellets were classified according to 4 standard sizes.

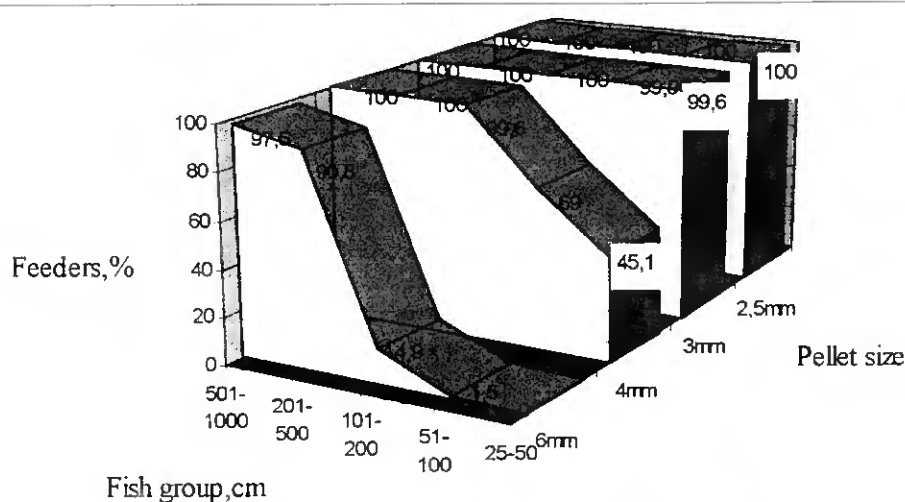


A correction factor, based on the Poisson Distribution, was introduced in order to estimate the “blind eaters” - fish which may have been eating pellets without label, considering the fact that, especially within the smaller pellets, not all of them had at least one Ballotini, as shown in **TABLE V**.

The probable rates of feeders if all the pellets were labelled, are shown in **FIGURE 10**:

- Large 6.0mm pellets were refused by the F.G. 1 and the number of feeders increases with fish dimensions among the remaining F.G.
- Pellets from 4.0mm, were eaten by all fish, but the number of feeders were greater among bigger fish.
- The 2.5mm and 3.0mm pellets, were eaten by almost all the fish population.

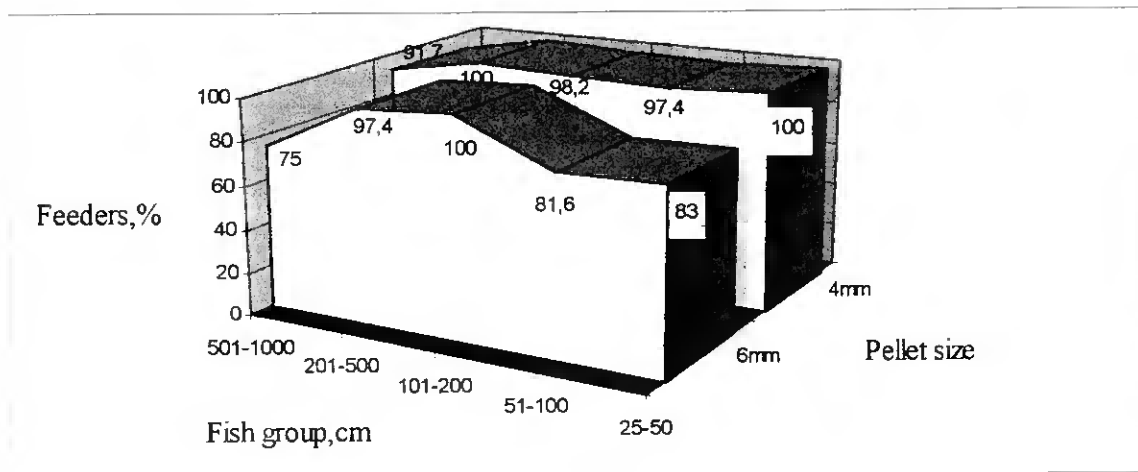
FIGURE 10 - Values represent the probability of trout (Feeders %) eating at least one pellet from "choice" treatment. Fish were divided in 5 fish size groups (g) and pellets were classified according to 4 standard sizes.



3.4. FEEDERS WITH "NO CHOICE"

No significant differences, among the 5 fish size groups, concerning the rate of feeders could be observed, when fish could not choose the pellet size, as shown in **FIGURE 11**.

FIGURE 11 - Trout (Feeders %) eating at least one pellet from “no choice” treatment. Fish were divided in 5 fish size groups (g) and pellets were classified according to 2 standard sizes.

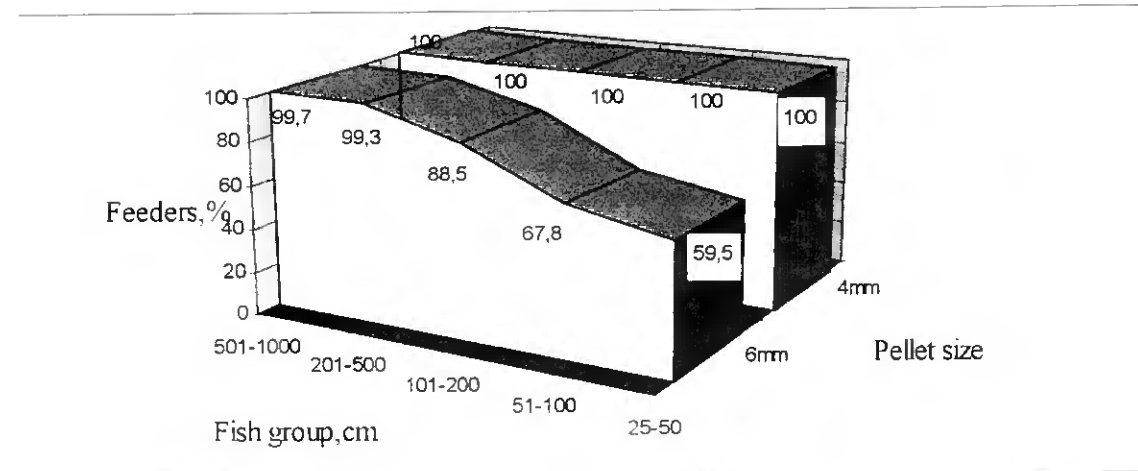


The same correction factor, based on the Poisson Distribution, was introduced in order to estimate the “blind eaters”.

The probable number of feeders eating large 6.0mm pellets, increased in proportion to weight of fish, from 60% to 100%, from trial with “no choice”, as shown from **FIGURE 12**.

Concerning the 4.0mm diet, it was observed that practically all of the fish has ingested at least one pellet.

FIGURE 12 - Values represent the probability of trout (Feeders %) eating at least one pellet from “no choice” treatment. Fish were divided in 5 fish size groups (g) and pellets were classified according to 2 standard sizes.



3.5. BIOMETRIC RELATIONSHIPS BETWEEN BODY AND MOUTH DIMENSIONS

The biometrics data among 55 random rainbow trout, the relationship within these values, for 4.0mm pellets, in trial with “choice”, were presented in **TABLE XI** and **FIGURES - 13, 14 and 15**.

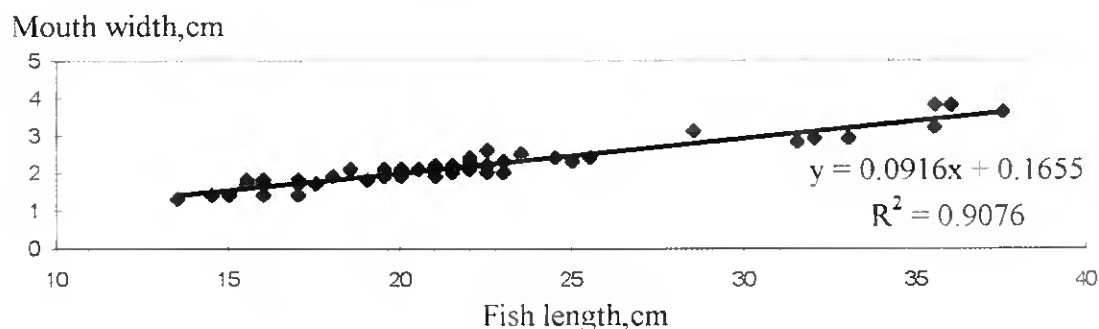
The biometrics average values among 55 random rainbow trout, divided into 5 fish size groups (F.G.) and the relationship between F.G, for 4.0mm pellets, in trial with “choice”, are shown in **TABLE XI**.

TABLE XI - The biometrics average values among 5 fish size groups of 55 random rainbow trout, for 4.0mm pellets, with “choice”.

Fish Weight Group (F.G.)	Average Fish Weight (F.W.), g	Fish Length (F.L.), cm	Mouth Width, cm	Body Section (F.W./F.L.), cm ²	Mouth Section, cm ²
1	42	14.0	1.3	3.0	1.4
2	73	16.6	1.7	4.4	2.3
3	153	21.2	2.1	7.2	3.5
4	299	26.6	2.6	11.0	5.2
5	717	35.5	3.5	20.1	9.5

The relationship between fish (fork) length and mouth gape (width), were analysed calculating regression (y) and a high coefficient of correlation ($R^2 = 0.91$), in 55 trout chosen at random, as shown in **FIGURE 13**.

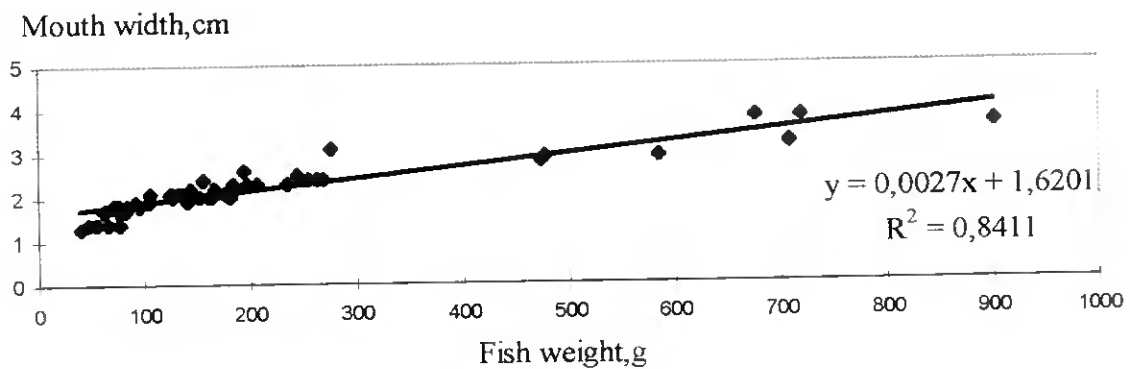
FIGURE 13 - Relationship between body (fork) length (cm) and mouth-width (cm), in 55 trout, chosen at random.



The formula of regression - y and coefficient of correlation - R^2 .

The relationship between fish weight (F.W.) and mouth gape (M.W.) and the correlation between them were analysed, calculating regression (y) and a high coefficient of correlation ($R^2 = 0.84$), in 55 trout chosen at random, as shown in **FIGURE 14**.

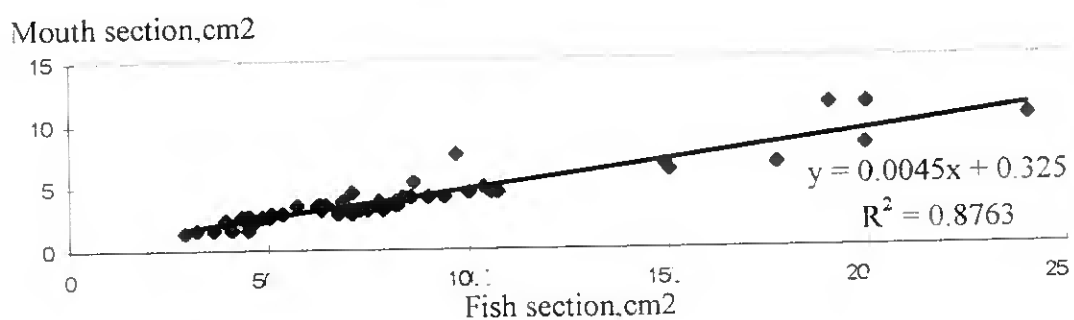
FIGURE 14 - Relationship between fish weight (g) and mouth-width (cm), in 55 trout, chosen at random.



The formula of regression - y and coefficient of correlation - R^2 .

The two dimensional measures, body sections (cm²) and mouth sections (cm²) were related, for a better interrelation among fish dimensions of different fish species and shapes. The correlation between them was analysed, calculating regression (y) and a high coefficient of correlation ($R^2 = 0.88$), in 55 trout chosen at random, are shown in **FIGURE 13**.

FIGURE 13 - Relationship between body-sections (cm²) of fish, and mouth-sections (cm²), in 55 trout, chosen at random.



The formula of regression - y and coefficient of correlation - R^2 .

4. DISCUSSION

Cho, (1992) comments, that feeding of fish continues to be an “art form” based on instinct and folklore, and regrets the few scientific studies of feeding standards.

The experiments described here, and carried out at Nutreco Research Centre, were intended to test the assumption that food pellet size is a determinant factor for food intake, and therefor successful fish farming. At present 16 pellet sizes for pellet sizes for rainbow trout are commercially available.

The initial experimental design consisted on trial “food intake with choice”. However, other subsequent trials took place, based on the observation of data, which could be done very rapidly, due to the versatility of the X-ray method described by Talbot & Higgins, (1982). The conclusions taken from the experiment were more conclusive after the introduction of the complementary trials “food intake with no choice”, “feeders” and “biometric relationships between body and mouth dimensions”.

4.1. FOOD INTAKE

The study of Interactions between feeding biology and feeding regimes is crucial for the success of fish farming and the appetite of fish is directly connected with growth and consequently with economic improvement in aquaculture (Talbot, 1994).

Food dimensions. is usually the strongest stimulus eliciting prey capture in adult fish (Kislalioglu and Gibson, 1976).

Considering the appetite of rainbow trout, between 25g and 200g, with possibility of choice for equal mixed weight of pellet diameters 2.5mm, 3.0mm, 4.0mm and 6.0mm, they preferred the two smaller pellet sizes. The opposite tendency was observed among large fish, between 201g and 1000g, which preferred large pellets - 4.0mm and 6.0mm, as shown in FIGURES 1, 2, 3 and 4.

However, when the size of food was not an option, and unmixed 4.0mm and 6.0mm pellet were tried, it could be observed that the total fish population (25-1000g) did not show significant differences on adjusted food intakes, as shown in FIGURES 5, 6, 7, and 8.

4.2. FEEDERS

In relation to the absolute upper limits for food size at ingestion, Wallace *et al.*, (1989), referred that it is not a good indicator of the size of food particles actually consumed by the fish. Experimental data show that few, if any, fish species constantly choose food particles of the maximum size ingestible.

Considering the total population of rainbow trout, between 25g and 1000g, with possibility of choice for equal mixed weight of pellet diameters 2.5mm, 3.0mm, 4.0mm and 6.0mm, the number of feeders decreased from 100% among total fish, till 0%, with smaller fish, when the 6.0mm diameter pellets were tried, as shown in FIGURE 10.

However, when the size of food was not an option, and unmixed 4.0mm and 6.0mm pellet were tried, it could be observed that, within the total fish population, the number of feeders decreased gradually from 100%, among total fish, till 83% (this observed percentage was considered, in this case, instead of the probable one - 60%, due to his higher value) with smaller fish, when 6.0mm pellets were tried and are shown in FIGURES 11 and 12.

From observation of data of 55 trout chosen at random, the relation between larger 6.0mm pellets diameter and average mouth width from smaller fish group (1.3cm), is near 1/2.

According to Egglshaw, (1967), wild salmon of 7cm length, had a higher food intake when the breadth of prey were near the mean mouth breadth.

Wankowski *et al.* (1979), in the laboratory, using salmonids of length between 2cm and 28cm came to a similar conclusion.

It appears that, for fish of 25g or more, mouth dimensions is not a limiting factor for ingestion of pellets up to 6mm diameter, as can be seen in **FIGURES 5 and 6**. One fish weighing 28g ingested at least 4 pellets of 6mm.

As was observed, from the trial with "choice", none of the fish were limited concerning food ingestion of pellet sizes 2.5mm and 3.0mm, it can be concluded from both complementary trials that no fish was limited in food intake or in ability to swallow any of the pellet sizes used.

In conclusion, when the size of food was not an option, it could be observed that the total fish population, at least between 28g (the smallest fish which swallowed 6.0mm pellets) and 1000g, did not show significant differences in intakes or biometrics limitations concerning ingestion of all pellet diameters 2.5mm, 3.0mm, 4.0mm and 6.0mm.

4.3. BIOMETRIC RELATIONSHIPS BETWEEN BODY AND MOUTH DIMENSIONS

The biometric relationships between mouth and body dimensions is considered relevant for a better knowledge of the limits of food-dimensions that fish of different sizes are able to ingest (Wallace *et al.*, 1989).

The three biometric relationships between body (fork) length / mouth gape (width); fish weight / mouth width; and body section / mouth section were analysed and, showed that the variation in body measures and within mouth gape are directly proportional.

Regression - $y = 0.09 x + 0.17$; and coefficient of correlation - $R^2 = 0.91$ were calculated, for the first biometrics study.

Wankowski *et al.*, (1979), for Atlantic salmon (*Salmo salar* L.) size range between 2cm and 28cm, has obtained similar results for relationship body fork length / mouth width: $y = 0.06 x + 0.05$ and $R^2 = 0.99$.

5. CONCLUSION

It is necessary to document, for each species and size of fish used in aquaculture, the range of food particle sizes capable of being ingested and those giving optimal growth and / or production (Wallace *et al.*, 1989).

The total rainbow trout population, between 25g and 1000g, did not show significant preferences or any biometrics limitations concerning ingestion of pellet diameters 2.5mm, 3.0mm, 4.0mm and 6.0mm.

The conclusions taken from this experiment, may help fish farmers and feed factories to reduce production costs, by eliminating unnecessary pellet sizes.

ANNEXE I - The Statistical Analysis of Variance (ANOVA) for adjusted food intake (F.I.a.); data were organised in 20 groups, where 5 fish size groups (F.G.) and 4 pellet diameters (P.S.) were related, for studies with "choice".

For F.I.a. c, classified by WG/PS.c

WG/PS.c	12.5	13	14	16
Size	36	35	26	24
Mean	22.5128722222	11.6626809143	2.21074484615	0
Median	19.14403	7.829819	0	0
Variance	328.783936569	187.836166658	19.3723755919	0
Standard deviation	18.1324001878	13.7053335114	4.40140609259	0
Standard error	3.02206669796	2.31662418592	0.86318675205	0
Coeff of variation	0.8054236709	1.17514434392	1.99091546012	0
Minimum	0	0	0	0
Maximum	95.87167	53.95009	15.02806	0
Range	95.87167	53.95009	15.02806	0
Lower quartile	11.3316	0	0	0
Upper quartile	29.83119	15.65964	3.633826	0
Interquart. range	18.49959	15.65964	3.633826	0
Skewness	2.08265828059	1.42419478813	2.05744718144	0
Std error skewness	0.39254393681	0.39769404433	0.45556022799	0.47226084215
Kurtosis	6.71909782602	1.77577622267	3.29656619151	0
Std error kurtosis	0.76807610663	0.7777943911	0.88650853007	0.9177770826
WG/PS.c	22.5	23	24	26
Size	58	72	58	78
Mean	28.5692657931	10.9397670972	3.13409491379	0.25410602564
Median	25.077375	9.389897	1.1169675	0
Variance	244.191777691	115.357938228	20.9839370499	5.03645003683
Standard deviation	15.6266368004	10.7404812848	4.58082274814	2.24420365315
Standard error	2.05187793585	1.26577785828	0.60149149462	0.25410602564
Coeff of variation	0.54697369241	0.98178335876	1.46160945158	8.83176086633
Minimum	2.537074	0	0	0
Maximum	65.88915	48.84277	17.64357	19.82027
Range	63.352076	48.84277	17.64357	19.82027
Lower quartile	16.89648	0	0	0
Upper quartile	42.02273	17.371905	4.710881	0
Interquart. range	25.12625	17.371905	4.710881	0
Skewness	0.29790010345	1.01230172548	1.80621840441	8.83176086633
Std error skewness	0.31371993256	0.28289805788	0.31371993256	0.2722108539
Kurtosis	-0.67472074136	0.93484209487	2.63744502633	78
Std error kurtosis	0.61813583683	0.55883121673	0.61813583683	0.53817641816
WG/PS.c	32.5	33	34	36
Size	54	48	64	62
Mean	20.4836680741	10.8472048958	7.93061734375	1.78909696774
Median	18.041255	8.242916	6.4524385	0
Variance	168.414670373	105.919202699	64.6663979092	15.1565315743
Standard deviation	12.9774677951	10.2917055292	8.04154201066	3.89313903866
Standard error	1.76600968063	1.48547973942	1.00519275133	0.49442915234
Coeff of variation	0.63355194725	0.94878870898	1.01398688931	2.17603579284
Minimum	0	0	0	0
Maximum	54.25223	41.50424	30.95384	16.47461
Range	54.25223	41.50424	30.95384	16.47461
Lower quartile	9.714718	2.653642	0.6197755	0
Upper quartile	27.77243	16.571705	11.98628	0
Interquart. range	18.057712	13.918063	11.3665045	0
Skewness	0.61808922942	1.06882055832	1.03984143367	2.37508513513
Std error skewness	0.32455626399	0.3431493092	0.2993270479	0.30390217564
Kurtosis	-0.12466185363	0.79261492589	0.42612828003	5.22712653297
Std error kurtosis	0.63889306916	0.67439742269	0.59049122523	0.59928801153

ANNEXE I (cont.)

WG/PS.c	42.5	43	44	46
Size	30	23	35	21
Mean	5.4428776	5.4918386087	14.1242012571	13.9386408095
Median	3.0048715	4.898949	11.63505	6.178193
Variance	37.3760385221	18.2339824443	99.3727734137	390.511683111
Standard deviation	6.11359456638	4.27012674803	9.9685893392	19.7613684524
Standard error	1.11618455048	0.89038297682	1.68499913873	4.31228413186
Coeff of variation	1.12322837581	0.77754046546	0.70578074878	1.41773998788
Minimum	0	0	0	0
Maximum	19.73938	13.45776	34.86091	90.6164
Range	19.73938	13.45776	34.86091	90.6164
Lower quartile	0	1.875253	6.787994	3.512049
Upper quartile	9.879219	9.064812	18.78212	18.08186
Interquart. range	9.879219	7.189559	11.994126	14.569811
Skewness	1.01789022776	0.28138724867	0.82667096777	3.13370706232
Std error skewness	0.42689239595	0.48133666148	0.39769404433	0.50119474483
Kurtosis	-0.05462605365	-1.13779530994	-0.32756336079	11.9226004889
Std error kurtosis	0.83274561836	0.93476379877	0.7777943911	0.97194102996

WG/PS.c	52.5	53	54	56
Size	20	20	17	15
Mean	5.34231015	4.66281035	11.0301289412	12.9577786667
Median	6.0527615	1.448643	3.24171	9.534215
Variance	24.9173163524	48.3963608046	190.98807409	110.201602299
Standard deviation	4.99172478732	6.95674929867	13.8198434901	10.4976950946
Standard error	1.11618359494	1.55557643343	3.35180437876	2.71049321833
Coeff of variation	0.93437570024	1.49196488308	1.25291767338	0.81014619594
Minimum	0	0	0	0
Maximum	17.69563	23.30346	49.91835	35.27511
Range	17.69563	23.30346	49.91835	35.27511
Lower quartile	0.235854	0	2.041677	5.382701
Upper quartile	8.6060555	6.4694305	16.73267	23.86027
Interquart. range	8.3702015	6.4694305	14.690993	18.477569
Skewness	0.64460235668	2.10090587182	1.70174996661	0.93411782811
Std error skewness	0.51210333671	0.51210333671	0.54974741675	0.58011935112
Kurtosis	0.12534827319	3.97726382506	2.86043724817	-0.18948557464
Std error kurtosis	0.99238361254	0.99238361254	1.06319782279	1.12089707664

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***** Classic Experimental Approach ANOVA *****

Dependent variable: FI.a. c

Due to	Sum of Squares	Deg Fre	Mean Square	F-stat	Signif
WG/PS.c	53482.082	19	2814.846	27.428	0.0000
Error	79639.677	776	102.628		
total	133121.758	795	167.449		

400 row(s) omitted due to missing values

ANNEXE II - Comparison of adjusted food intakes (F.I.a.) within 20 groups, where 5 fish size groups (F.G.) and 4 pellet diameters (P.S.) were related, by Duncan's Multiple Range Test (95% confidence limit), obtained from studies with "choice" feeding.

Dependent variable: FI.a. c

Method:95% Duncan interval.

Table Ranges: 2.78 2.93 3.01 3.09 3.15 3.2 3.24 3.28 3.3 3.33 3.35 3.37 3.38 3.4
3.41 3.42 3.45 3.46 3.46

* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	16	26	36	14	24	53	52.
16	24	0							
26	78	0.25410602564							
36	62	1.78909696774							
14	26	2.21074484615							
24	58	3.13409491379							
53	20	4.66281035							
52.5	20	5.34231015							
42.5	30	5.4428776		*					
43	23	5.4918386087							
34	64	7.93061734375	*	*	*	*	*		
33	48	10.8472048958	*	*	*	*	*	*	
23	72	10.9397670972	*	*	*	*	*	*	
54	17	11.0301289412	*	*	*	*	*	*	
13	35	11.6626809143	*	*	*	*	*	*	
56	15	12.9577786667	*	*	*	*	*	*	*
46	21	13.9386408095	*	*	*	*	*	*	*
44	35	14.1242012571	*	*	*	*	*	*	*
32.5	54	20.4836680741	*	*	*	*	*	*	*
12.5	36	22.5128722222	*	*	*	*	*	*	*
22.5	58	28.5692657931	*	*	*	*	*	*	*

Method:95% Duncan interval.

Table Ranges: 2.78 2.93 3.01 3.09 3.15 3.2 3.24 3.28 3.3 3.33 3.35 3.37 3.38 3.4
3.41 3.42 3.45 3.46 3.46

* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	42.	43	34	33	23	54	13
16	24	0			*	*	*	*	*
26	78	0.25410602564	*		*	*	*	*	*
36	62	1.78909696774			*	*	*	*	*
14	26	2.21074484615			*	*	*	*	*
24	58	3.13409491379			*	*	*	*	*
53	20	4.66281035				*	*		*
52.5	20	5.34231015							*
42.5	30	5.4428776				*	*		*
43	23	5.4918386087				*	*		*
34	64	7.93061734375							
33	48	10.8472048958	*	*					
23	72	10.9397670972	*	*					
54	17	11.0301289412							
13	35	11.6626809143	*	*					
56	15	12.9577786667	*						
46	21	13.9386408095	*	*	*				
44	35	14.1242012571	*	*	*				
32.5	54	20.4836680741	*	*	*	*	*	*	*
12.5	36	22.5128722222	*	*	*	*	*	*	*
22.5	58	28.5692657931	*	*	*	*	*	*	*

ANNEXE II (cont.)

Method:95% Duncan interval.

Table Ranges: 2.78 2.93 3.01 3.09 3.15 3.2 3.24 3.28 3.3 3.33 3.35 3.37 3.38 3.4
3.41 3.42 3.45 3.46 3.46

* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	56	46	44	32.	12.	22.
16	24	0	*	*	*	*	*	*
26	78	0.25410602564	*	*	*	*	*	*
36	62	1.78909696774	*	*	*	*	*	*
14	26	2.21074484615	*	*	*	*	*	*
24	58	3.13409491379	*	*	*	*	*	*
53	20	4.66281035	*	*	*	*	*	*
52.5	20	5.34231015	*	*	*	*	*	*
42.5	30	5.4428776	*	*	*	*	*	*
43	23	5.4918386087		*	*	*	*	*
34	64	7.93061734375		*	*	*	*	*
33	48	10.8472048958				*	*	*
23	72	10.9397670972				*	*	*
54	17	11.0301289412				*	*	*
13	35	11.6626809143				*	*	*
56	15	12.9577786667				*	*	*
46	21	13.9386408095				*	*	*
44	35	14.1242012571				*	*	*
32.5	54	20.4836680741	*	*	*			*
12.5	36	22.5128722222	*	*	*			*
22.5	58	28.5692657931	*	*	*	*	*	*

ANNEXE III- The Statistical Analysis of Variance (ANOVA) for adjusted food intake (F.I.a.); data were organised in 10 groups, where 5 fish size groups (F.G.) and 2 pellet diameters (P.S.) were related, for studies with "no choice".

For FI.a nc, classified by WG/PSnc

WG/PSnc	14	16	24	26
Size	32	47	76	61
Mean	67.315795625	25.1089285106	55.7922125	23.8518992623
Median	50.673385	20.84746	54.694435	20.50773
Variance	2781.80414141	443.993223307	758.494704871	383.82312735
Standard deviation	52.7428112771	21.0711467013	27.5407825755	19.5914044252
Standard error	9.32369987822	3.07354263443	3.15914442296	2.5084222961
Coeff of variation	0.783513153	0.83918940198	0.49363130339	0.8213771243
Minimum	21.39199	0	0	0
Maximum	231.9449	84.68945	186.2908	67.83799
Range	210.55291	84.68945	186.2908	67.83799
Lower quartile	39.23884	10.26698	41.161525	8.633061
Upper quartile	70.413405	41.06792	67.80422	34.53225
Interquart. range	31.174565	30.80094	26.642695	25.899189
Skewness	2.48557098278	0.85798489164	2.02122184609	0.67394946993
Std error skewness	0.41445734615	0.34657049948	0.275637489	0.30626990959
Kurtosis	5.53877530989	0.43180745798	8.62931992162	-0.29329161428
Std error kurtosis	0.80937128681	0.68091533075	0.54480406	0.60383715373
WG/PSnc	34	36	44	46
Size	57	50	23	38
Mean	58.1530173684	25.37042644	57.7651678261	37.6894171053
Median	57.46863	22.35862	63.93572	35.289455
Variance	484.247727331	236.98008199	845.65755311	375.648483888
Standard deviation	22.0056294464	15.3941573979	29.0801917654	19.3816532806
Standard error	2.91471682393	2.17706261734	6.06363914662	3.14411933904
Coeff of variation	0.37840907389	0.60677566592	0.50342088251	0.51424656493
Minimum	0	4.271209	14.9237	0
Maximum	122.9197	64.06814	132.6395	82.80778
Range	122.9197	59.796931	117.7158	82.80778
Lower quartile	42.48717	15.9737	29.8474	23.10728
Upper quartile	69.38877	30.26072	74.89415	52.87057
Interquart. range	26.9016	14.28702	45.04675	29.76329
Skewness	0.51502822328	1.01656252266	0.47455824888	0.42844097943
Std error skewness	0.31632688145	0.33660070855	0.48133666148	0.38281839955
Kurtosis	1.24847012752	0.40568413909	0.59439395141	-0.53729467386
Std error kurtosis	0.62313390362	0.66190837451	0.93476379877	0.7497003516
WG/PSnc	54	56		
Size	12	4		
Mean	52.5472693333	23.353765		
Median	62.24412	25.291355		
Variance	821.331262822	344.265077398		
Standard deviation	28.6588775569	18.5543816226		
Standard error	8.27310533608	9.27719081132		
Coeff of variation	0.5453923281	0.7944920925		
Minimum	0	0		
Maximum	90.77096	42.83235		
Range	90.77096	42.83235		
Lower quartile	36.73206	9.10179		
Upper quartile	70.31667	37.60574		
Interquart. range	33.58461	28.50395		
Skewness	-0.90200490305	-0.49554735884		
Std error skewness	0.63730200545	1.01418510567		
Kurtosis	-0.01745346501	-0.87878237482		
Std error kurtosis	1.23224647394	2.61861468283		

ANNEXE III (cont.)

***** Classic Experimental Approach ANOVA *****

Dependent variable: FI.a nc

Due to	Sum of Squares	Deg Fre	Mean Square	F-stat	Signif
WG/PSnc	106092.238	9	11788.026	17.162	0.0000
Error	267876.903	390	686.864		
total	373969.141	399	937.266		

796 row(s) omitted due to missing values

ANNEXE IV - Comparison of adjusted food intakes (F.I.a.) within 10 groups, where 5 fish size groups (F.G.) and 2 pellet diameters (P.S.) were related, by Duncan's Multiple Range Test (95% confidence limit), obtained from studies with "choice" feeding.

Dependent variable: FI.a nc

Method:95% Duncan interval.

Table Ranges: 2.79 2.93 3.02 3.09 3.15 3.2 3.24 3.28 3.31

* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	56	26	16	36	46	54	24
56	4	23.353765							*
26	61	23.8518992623					*	*	*
16	47	25.1089285106					*	*	*
36	50	25.37042644					*	*	*
46	38	37.6894171053		*	*	*			*
54	12	52.5472693333		*	*	*			
24	76	55.7922125	*	*	*	*	*		
44	23	57.7651678261	*	*	*	*	*		
34	57	58.1530173684	*	*	*	*	*		
14	32	67.315795625	*	*	*	*	*		

Method:95% Duncan interval.

Table Ranges: 2.79 2.93 3.02 3.09 3.15 3.2 3.24 3.28 3.31

* denotes significantly different pairs. Vertical bars show homogeneous subsets.

group	cases	mean	44	34	14
56	4	23.353765	*	*	*
26	61	23.8518992623	*	*	*
16	47	25.1089285106	*	*	*
36	50	25.37042644	*	*	*
46	38	37.6894171053	*	*	*
54	12	52.5472693333			
24	76	55.7922125			
44	23	57.7651678261			
34	57	58.1530173684			
14	32	67.315795625			

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