ABSTRACT OF PHD THESIS

Trout meat is a food with high biological value, due to its important content in protein, easily digestible and without secondary effects unfavourable for human health; also being a source of raw materials for different domains of industry.

Research carried out focused mainly on creation of a large image on rainbow trout (*Oncorhynchus mykiss)* and brook trout (*Salvelinus fontinalis)*, being approached problems regarding physiology, internal immune-biochemical processes, metrical characters and rearing technologies specify to those two breeds.

In our research we aim to realise a comparative analysis between biochemical and metrical characteristics of trout from two different rearing systems (semi-intensive and intensive). Semi-intensive salmonid basins are constructions with own alimentation and evacuation systems. This system is specific to the majority of trout farms with ground ponds in Romania; intensive systems are the most utilised ones in Romania, rearing ponds varied very much as shape and size, function of breed, age category and structure of terrain.

Those ponds are designed function of biological demands for each age: rearing density, individuals' dimensions, foddering level.

The own contribution include a biometrical analysis of gathered individuals from different farms, so through correlation with the existent characteristics and environment factors to be establish similarities and differences with a high trusting level between salmonidae. Also we aimed on a complex biochemical analysis of tissues gathered from salmonidae to be able to establish which of the environmental factors, food, age have an influence on biochemical characteristics of analysed salmonids. By multi-factorial analysis we aimed to enlightened factor or factors which must be best controlled in artificial environment and which are the differences face to other cases presented in literature. So, research regarding biochemistry of rainbow and brook trout is useful in presenting the importance of different carbohydrate, lipid, protein and enzymatic characteristics as biomarkers for influence of different food types, environmental conditions or pollution. All of those highlight the importance of biochemical research which were carried out, research correlated with biometrical and sensorial ones.

Research was focused on three directions: 1. study of literature in library and consultation of data bases available for Faculty of Animal Sciences (UASVM Iaşi) and Faculty of Biology ("Al. I. Cuza" University from Iaşi); 2. field works and gathering of samples which were subjected to analysis in laboratory; 3. analysis of gathered samples in certified laboratories.

The own research were carried out on four series of experiences and aimed to create an objective image on morphological, productive and functional characteristics of cultured salmonidae, in different

stages of growing and development.

The four experimental series are represented by:

- 1st series of experiences – Dates regarding management of salmonidae rearing;
- 2nd series of experiences – Obtained results regarding knowledge of salmonids' biology and physiology;
- 3rd series of experiences – Obtained results regarding knowledge of sensorial, physical-chemical and microbiological features of trout meat;
- 4th series of experiences – Obtained results regarding some functional characteristics of cultured salmonids.

Biological material was represented by rainbow trout (*Oncorhynchus mykiss*), respectively brook trout (*Salvelinus fontinalis*), reared in two salmonidae exploitations (Stejaru trout farm - E1 and Cheiţa trout farm - E2) designed for touristic and productive purposes from Neamţ County. To achieve the research goals were establish six experimental batches marked PC_2, PC_3, PC_4, PF_2, PF_3, PF_4 formed by 30 individuals per batch, on three age categories.

Salvelinus fontinalis (brook trout) is a breed originally from the region of the river springs from Atlantic coast of USA, being introduced in Romania around year 1906, being considered one of the most beautiful mountain fishes in Romania, due to its colours harmony. Brook trout manifest a remarkable exigency face to water temperature, which must not be below 4ºC and not to over-pass 15ºC. Feeding activity is more intense between 12 and 14ºC, consuming in generally, snails, earthworms, insect larvae and insects from water surface. Brook trout have a more rapid growing face to indigenous trout, so at the age of two years could reach a weight of 200 g.

Oncorhynchus mykiss (rainbow trout) is originally from USA, being introduced in Europe in 1880, and later being imported in Romania, in Moldova area. Rainbow trout is less exigent at physical-chemical qualities of water, in comparison with all salmonids. This breed endures quite easily murky waters, but for short periods of time, as well as the hottest waters, but with great flows and quite rich in dissolved oxygen. This fish efficiently feeds at a water temperature between 15 and 19ºC, but stops the feeding at over 23ºC; is characterized by an intense rhythm of corporal growing, reaching at the age of two years a weight of 250 g.

In *feeding* of cultured trout is utilised, in the last period of time, concentrated food represented by granulated mixed fodders, expanded or extruded, with a cylindrical shape and different dimensions function of fish age.

In exploitation E2-Cheiţa foddering of trout is manually realised, with extruded granulated fodders, with a protein level between 42 and 50% crude protein. Food is administrated in 1-2 meals per day function of season, and food daily quantity is administrated function of water temperature and represents between 0.5 and 1.4% from corporal mass of trout.

In Stejaru trout farm population densities are lower than in intensive system (Cheiţa trout farm), trout being feed with granulated fodders, but is not excluded their feeding with natural food. Foddering of trout is manually made, with extruded granulated fodders, with a protein level between 40–45% crude protein.

Food is administrated in only one meal per day, and daily food quantity which is administrated function of fish corporal mass and water temperature, represents up to 1.8% from trout corporal mass.

In our research samples' gathering, measurements, gravimetric determinations, physical-chemical determinations and the immune-biochemical ones were realised using different types of apparatuses, utensils, kits for biochemical determinations and reagents.

To calculate growing indexes and coefficients were determined the following parameters: total length, standard length, head length, caudal peduncle length, maximal height of body and maximal thickness of body. Based on somatic measurements will be calculated a series of corporal indexes, which provide information regarding maintenance state and corporal shape of fishes.

Regarding *weight increasing dynamics* the best results were recorded at trout individuals from batches PF_4 and PC_4, at which growing gain per individual and period was of 88.53–101.04 g, net superior to the ones of individuals from batches PF_3 and PC_3 (59.19–72.45 g) and especially face to individuals from batches PF_2, PC_2 (42.35–62.52 g).

Regarding the corporal dimensions for brook trout individuals from Stejaru trout farm (E1) were obtained the following mean values: body mass = 252.20 g; standard length (ls) = 24.51 cm, maximal height of body (H) = 7.03 cm, head length (lc) = 5.42 cm, maximal circumference of body (C) = 17.01 cm, length of caudal peduncle (lp) = 4.90 cm and maximal thickness of body (G) = 3.07 cm.

Individuals of brook trout gathered from Cheiţa trout farm (E2) recorded mean values of 279.75 g for body mass, 25.23 cm for standard length (ls), 7.16 cm for maximal height of body (H), 5.40 cm for head length (lc), 16.84 cm for maximal circumference of body (C), 5.26 cm for length of caudal peduncle (lp) and 3.09 cm for maximal thickness of body (G).

Rainbow trout from salmonids' exploitation Stejaru (E1) had a body mass of 263.98 g, standard length (ls) of 24.66 cm, maximal height of body (H) was 7.0 cm, head length (lc) of 5.12 cm, maximal circumference of body (C) was 17.38 cm, length of caudal peduncle (lp) was 4.93 cm and maximal thickness of body (G) was 3.17 cm.

At Cheiţa trout farm (E2), studied individuals of rainbow trout recorded the following values: body mass – 276.71 g; standard length (ls) – 25.19 cm; maximal height of body (H) – 6.93 cm; head length (lc) – 5.24 cm; maximal circumference of body (C) – 17.61 cm; length of

caudal peduncle (lp) – 5.21 cm and maximal thickness of body (G) – 3.21 cm.

After weighting rainbow trout individuals resulted that mean values for corporal mass were of 263.98±5.97 g at Stejaru trout farm (E1) and mean values of 276.71±6.32 g were obtained at Cheiţa trout farm (E2). For brook trout mean values of corporal mass were of 252.20±11.98 g at Stejaru trout farm (E1) and of 279.75±7.70 g at Cheiţa trout farm (E2).

Effectuated measurements on brook and rainbow trout enlightened close values to the ones presented in literature, fact which demonstrates that in both studied units were assured optimal conditions for growing and development.

Based on the external corporal variables we carried out an *analysis of morphological characters*, as well as the calculation of corporal indexes and coefficients for both studied trout breeds.

Total length of body was greater with 11.88% at individuals from breed *Oncorhynchus mykiss* face to the ones belonging to *Salvelinus fontinalis* breed (27.36 cm vs. 24.11cm).

Standard mean length of body presented values a little bit higher for rainbow trout (24.74±3.48 cm) in comparison with brook trout (22.13±3.08 cm).

Regarding the *mean length of caudal peduncle*, individuals of *Oncorhynchus mykiss* (rainbow trout) recorded a mean value of 5.13±0.87 cm, and the ones from *Salvelinus fontinalis* breed (brook trout) of 4.88±0.72 cm.

Related to those three analysed stages of corporal development, were enlightened higher values for *mean height* and *circumference of body* at population of *Oncorhynchus mykiss* (rainbow trout). Mean thickness of body at individuals belonging to breed *Oncorhynchus mykiss* were placed at a mean value of 3.18±0.56 cm, face to 3.04±0.53 cm as was at *Salvelinus fontinalis* (brook trout).

Function of rearing system of studied salmonidae, mean values of analysed corporal variables for individuals grown in semi-intensive system were lower than at the ones grown in intensive system.

At *Oncorhynchus mykiss* breed (rainbow trout) *with age of two years* were recorded higher values for the main corporal variables at individuals reared in intensive system face to the ones from semi-intensive system. So, for total length, standard length, head length, length of caudal peduncle, height and thickness of body were identified very significant differences ($p<0.001$); and for body circumference distinct significant differences ($p<0.01$).

Individuals from *Salvelinus fontinalis* breed (brook trout) *with age of two years* recorded very significant differences ($p<0.001$) for total length, standard length, head length, length of caudal peduncle, circumference and thickness of body and differences without signification ($p>0.05$) for body height, between those two studied rearing systems.

At *rainbow trout with an age of three years*, total length, standard length, head length, height and thickness of body didn't present significant differences ($p>0.05$) between those two analysed rearing systems; distinct significant differences ($p<0.01$) were founded for body circumference, and very significant differences ($p<0.001$) for caudal peduncle length.

For *brook trout with age of three years*, total length, standard length, caudal peduncle length, height and circumference of body presented very significant differences ($p<0.001$), and head length and body circumference only distinct significant differences ($p<0.01$), between those two rearing systems.

At individuals of *rainbow trout with age of four years* were identified very significant statistical differences ($p<0.001$) in the case of total length, standard length, caudal peduncle length, body height and circumference, and also their missing ($p>0.05$) in the case of head length and body thickness.

At individuals from *Salvelinus fontinalis* breed (brook trout) *with age of four years* were recorded insignificant differences ($p>0.05$) for heads' length, while for the rest of corporal variables statistical differences were very significant ($p<0.001$), at comparison of those two rearing systems.

Profile index at individuals from batch PF_2 recorded a mean value of 3.50±0.06 in exploitation E1-Stejaru, and of 3.53±0.08 at the ones from exploitation E2-Cheiţa. Batch PC_2 had a profile index of 3.54±0.09 cm at individuals from exploitation E1-Stejaru and 3.66±0.09 cm at the ones from exploitation E2-Cheiţa. These indicate a harmonious corporal format in the case of all four experimental batches.

Quality index or Kiselev (IC). Trout from exploitation E1-Stejaru presented the lowest mean value for quality index, of only 1.42±0.03 cm, and at the ones from exploitation Cheiţa-E2, the highest value was 1.50±0.03 cm. We remark the very good homogeneity of the analysed character, values of variation coefficient being under 10% (V%= 4.35–9.47).

Values of *Fulton coefficient* oscillated in interval 1.28±0.03 (Exploitation Stejaru-E1) and 1.36±0.05 (Exploitation Cheiţa-E2). Variability of this character was lower inside exploitation Stejaru-E1, where values for variability coefficient were between 6.23 and 7.66; and average homogenous in exploitation Cheiţa-E2, obtained values being between 14.62 and 16.08.

Carnosity index recorded a minimum of 20.83±0.31% at rainbow trout from exploitation Cheiţa-E2 and a maximum of 22.13±0.63% at brook trout from exploitation Stejaru-E1. Studied character was homogenous for all four trout batches, aspects enlightened by the value of variation coefficient which oscillated in interval 6.65–9.04%.

As a fact that values of carnosity index oscillates around the value of 20%, certify that studied trout had a suitable corporal development, are healthy and have a high percent of meat.

In case of cultured brook trout, the values for *hot efficiency* were of 79.86±0.32% in exploitation E1 and respectively of 79.82±0.17% in exploitation Cheiţa-E2; for cultured rainbow trout, the same feature had values between 82.49±0.16% exploitation E1 and 82.24±0.20% in exploitation Cheiţa-E2.

Cold efficiency was determined after previous refrigeration at trout carcasses for 24 hours, at a temperature between 1 and 1.5ºC. Values of cold efficiency for cultured brook trout were in the interval 77.82±0.32 – 78.75±0.15%, and at cultured rainbow trout the same characteristic had values between 81.49±0.14% and 82.23±0.18%.

Weighting of carcasses gathered from brook and rainbow trout individuals from all four batches enlightened that after refrigeration those ones lost between 1.5 and 2.0% from the initial weight, being in the limits cited in the literature.

Study of *quantitative particularities of meat production* at breeds *Salvelinus fontinalis* (brook trout) and *Oncorynchus mykiss* (rainbow trout) included also the establishment of internal organs mass (liver, kidneys and heart); as well as visceral mass.

Weight of visceral mass at studied trout was between 27.87±0.91 g and 28.19±0.83 g at brook trout individuals respectively between 37.77±1.36 g and 39.65±0.99 g at rainbow trout individuals. Analysing the mass of those three internal organs which were studied, we notice that the highest rate belonged to liver (4.04±0.11% at *Oncorynchus mykiss*-rainbow trout), and the lowest one belonged to heart (0.19±0.01 and 0.29±0.01% from the total corporal mass of studied trout).

At breed *Oncorhynchus mykiss* (rainbow trout) head rate (after removing of gills) was between 8.81–8.95%, body weight related to carcass weight presented very good values being in the interval 88.96% and 89.29%, and rate of fins represented 1.76-2.23% from carcass weight.

In case of breed *Salvelinus fontinalis* (brook trout), head rate had values between 8.56–8.76%; body weight related to carcass weight presented good values of 89.06% and 89.66%, and rate of fins was between 1.78 – 2.18% from carcass weight.

Regarding *sensorial properties of meat* in the case of all studied fishes, meat presented at surface a film with a white-rose colour with a light shade of grey, with a firm and elastic consistency, with a pleasant small, and characteristic for breed. In conclusion, sensorial parameters of analysed trout show the fact that those ones are optimal for consumption, due to their suitable freshness state.

pH value was determined on fresh meat and respectively after 24 from slaughtering, recording the following levels:

- 6.97 and 7.12 after slaughtering and at 24 hours between 6.79 and 6.82 for rainbow trout;
- 6.99 and 7.19 after slaughtering and at 24 hours between 6.87 and 6.90 for brook trout.

Values of *easily hydrolysable nitrogen* just after slaughtering varied between 19.11 mg/100 g at rainbow trout and 19.13 mg/100 g at brook trout. This indicator progressively increased during storage period, in parallel with depreciation of the qualities of analysed trout, so they became improper for consumption after eight days of keeping at temperatures of 2–4ºC, when values for easily hydrolysable nitrogen were 33.62 mg/100 g at rainbow trout and 34.61 mg/100 g at brook trout. At the end of refrigeration period the values for this parameter were 37.33 mg/100 g at rainbow trout and 37.43 mg/100 g at brook trout (altered meat).

Through analysis of Eber, Nessler reactions and of *sulphide hydrogen* we conclude that period in which trout meat could be kept in refrigeration conditions is of 3-4 days, after that becoming improper for human consumption.

Due to the fact that samples' gathering and processing was executed in optimal hygiene conditions and at short time after fishing, *meat microbiological charge* recorded low values between 1.02×10^3 and 1.08×10^3 NTG/UFC/g.

Meat content in collagen recorded values of 3.81–4.32% at rainbow trout and of 3.97–4.42% at brook trout, being in the limits given by the literature. Low rate in collagen of fish meat indicates that these one is easier to be cooked in comparison with other types of meat.

From *glucose* analysis resulted values of 19.3–23.1 mg/dl at individuals of *Oncorhynchus mykiss* - rainbow trout and of 19.1–22.9 mg/dl at individuals of *Salvelinus fontinalis* - brook trout, from where the lack of significant differences ($p>0.05$) between the obtained mean values for those two salmonidae.

Dosage of *glycogen* and comparative analysis between *Oncorhynchus mykiss* (rainbow trout) and *Salvelinus fontinalis* (brook trout) from semi-intensive rearing system, indicated the lack of statistical differences ($p>0.05$), both for hepatic glycogen (1.992±0.758 g/100g vs. 1.557±1.020 g/100g); and also for muscular glycogen (0.095±0.015 g/100g vs. 0.094±0.018 g/100g).

Values of hepatic and muscular glycogen at those two breeds (*Oncorhynchus mykiss* - rainbow trout, *Salvelinus fontinalis* - brook trout) reared in intensive system presented very significant differences ($p<0.001$) (2.325±0.868 g/100g vs. 3.832±0.438 g/100g and respectively 0.098±0.016 g/100g vs. 0.130±0.042 g/100g).

Mean values for *protein* at individuals of *Oncorhynchus mykiss* - rainbow trout and *Salvelinus fontinalis* - brook trout indicated an uniform activity; being situated in interval 0.173–0.213 g/dl (rainbow trout) and 0.169–0.209 g/dl (brook trout), and the difference couldn't be statistically covered ($p>0.05$).

Activity of *α-amylase* from digestive tube was between limits 10.9–15.3 U/l for rainbow trout and between 11.2–16.0 U/l for brook trout; mean values of this parameter (13.13±0.44 U/l

vs. 13.63±0.48 U/l) didn't present differences from statistical point of view ($p>0.05$).

Determination of intestinal *lipase* activity enlightened large variations from one individual to another, being between 0.07-0.20 U/l at rainbow trout and 0.27-0.39 U/l at brook trout. Mean values of those two analysed salmonids breeds (0.13±0.12 U/l vs. 0.33±0.12 U/l) leaded to apparition of very significant statistical differences ($p<0.001$).

Even if *alanine-aminotransferase* recorded maximum levels of 17.00 U/l at rainbow trout and of 16.00 U/l at brook trout; mean values enlightened lack of statistical differences between those two trout breeds (9.44±5.22 U/l vs. 9.05±5.10 U/l).

Activity of *aspartate-aminotransferase* from digestive tube of studied trout presented accentuated individual variations, oscillating between 5.00-132.00 U/l for rainbow trout and 5.00-130.00 U/l for brook trout; nevertheless, didn't existed significant differences ($p>0.05$) for activity of aspartate-aminotransferase between those two breeds (63.33±4.19 U/l vs. 63.50±4.15 U/l).

Quantitative determination of studied hormones was realised through ELISA method (*Enzyme-linked Immunosorbent Assay*) "sandwich" type.

Obtained results at the end of analysis for *growing hormone (hGH)* show insignificant differences ($p>0.05$) from statistic point of view between those two analysed trout breeds (0.511±0.40 ng/ml rainbow trout vs. 0.698±0.23 ng/ml brook trout).

Cortisol dosed at *unstressed trout* batch recorded values of 87.50±7.41 µg/dl for rainbow trout and of 81.27±3.05 µg/dl for brook trout; between those two batches statistical differences were very significant ($p<0.001$).

Due to the action of stress factors, sanguine samples which were analysed presented much more higher values for cortisol both at *stressed individuals "0h"* (164.60±18.36 µg/dl – rainbow trout; 160.80±14.76 µg/dl – brook trout), as well as at *stressed individuals "1h"* (295.55±14.73 µg/dl for rainbow trout and 298.06±8.30µg/dl for brook trout). Even if between the batches subjected to stress ("0h", respectively "1h") didn't existed differences with statistical signification ($p>0.05$), between batch with unstressed trout and the one with stressed trout the differences were very significant ($p<0.001$).

Based on the above mentioned conclusion we could make a series of *recommendations*:

- Application of the principles of intensive rearing system in salmoniculture, because assure higher productions on square unit in comparison with semi-intensive system;
- Rearing and exploitation of rainbow trout (*Oncorhynchus mykiss)* in the units from studied area because realise morph-productive indexes superior to the ones of brook trout (*Salvelinus fontinaliss)*;

- Capitalization on market of trout with age of two years due to the affordable rate between production cost and selling price;
- Trout delivery to beneficiary to be made immediately after gathering due to the fact that in this moment it is the lowest hormonal and biochemical products discharge, which could affect the integrity characteristics specific to trout meat;
- Trout storage (in production unit or at merchants) to be done in refrigeration regine and on a period of maximum 4 days.