

RESEARCH REGARDING THE INFLUENCE OF REARING CONDITIONS ON GLYCOGEN QUANTITY AT RAINBOW AND BROOK TROUT BREEDS REARED IN CHEIȚA TROUT FARM FROM NEAMȚ COUNTY, ROMANIA

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Abstract

The current paper aimed to study the influence of artificial rearing conditions on glycogen quantity for two trout breeds – rainbow trout (*Oncorhynchus mykiss* - Walbaum, 1792) and brook trout (*Salvelinus fontinalis* - Richardson, 1836), analysing 40 individuals, 20 individuals from each breed, belonging to Cheița trout farm from Neamț County. The current research is a part of an ample study designed to enlighten the differences of nutritional value for human consumption of trout meat reared in natural and artificial conditions. Trout represent also an important source of carbohydrates, through its reserves of hepatic and muscular glycogen. Glycogen is synthesised in animal organism by the mono-carbohydrates supplied by food, and in the case of an insufficient contribution of alimentary carbohydrates, it is formed from the resulted products of lipids and proteins catabolism. The obtained data resulted at the end of effectuated analysis on hepatic tissue record mean values of 2.325 ± 0.892 g/100g for rainbow trout and of 3.832 ± 0.450 g/100g for brook trout. In the case of muscular tissue were recorded mean values of 0.098 ± 0.016 g/100g for rainbow trout and 0.130 ± 0.044 g/100g for brook trout. Non-uniformity of biological material as regarding the corporal development and implicit of the tracked carbohydrates parameters have a great influence on the results, increasing in a considerable way their variability degree.

Key words: hepatic glycogen, muscular glycogen, *Oncorhynchus mykiss*, *Salvelinus fontinalis*

INTRODUCTION

Glucose occupies a central role in metabolism, also as a fuel and as a forerunner of essential structural carbohydrates and other bio-molecules. The role of carbohydrates in the living world is a main one given by the importance, amplitude and multitude of processes which took place in living organisms and helps in life maintaining. So, the main role of carbohydrates is the one of energy reserve. Animals could consume carbohydrates which will be oxidised to provide energy utilised in metabolic processes (Moran, L.A., et al., 2012, Tănăsescu, Gh., et al. 1973).

Beside the energetic role, carbohydrates participate to other fundamental biological functions such as nuclear function, under the form of pentose from the structure of nucleic acids; supportive function, through conjunctive tissue which have in its composition high quantities of mucopolysaccharides; nerve function, by participation of some substances with a complex lipid nature, and in its composition could be found glucose, galactose or oligoside; detoxification function, through glucuronic acid, derived by oxidation of glucose (Tănăsescu, Gh., et al. 1973).

At human and vertebrates, two carbohydrates substances have an important physiological importance: glycogen, condensed polymer as reserved carbohydrate material and glucose, as circulating form, easy to be metabolised by cellular systems

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and with a dominant energetic role (Tănăsescu, Gh., et al. 1973).

Liver and muscular tissues are the two major tissues for glycogen depositing. Liver capacity to store glycogen is sufficient to furnish to brain a glucose quantity for almost half a day in conditions of alimentary repose or malnutrition. When the level of glucose from blood decrease during fasting period the breakdown through glycogenolysis of glycogen stored in liver is the main defence line for preventing hypoglycaemia (Storey, K.B., 2004).

Glycogen represents the main energy reserve for satisfaction of metabolic demands on short term in the tissues of animal organisms. Glycogen content could reflect some biochemical adaptation of any stress forms given by the environment. From those ones pH, oxygen level, salinity and prolonged physical activity directly affect the glycogen reserves.

Starting from this premises the current paper aimed to determine the total glycogen quantity (g/100g) from white musculature and hepatic tissues gathered from 40 individuals of rainbow (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) reared in intensive growing system at Cheița trout farm, Neamț County.

MATERIAL AND METHODS

For a quantitative determination of glycogen research was carried out on biological material represented by white musculature and hepatic tissue of some trout from *Oncorhynchus mykiss* breed (rainbow trout) and *Salvelinus fontinalis* (brook trout). Fishes are collected from Cheița trout farm, Neamț County. Trout farm have as a main goal rearing of trout for consumption and spawn for populating and repopulating.

Animal tissue is subjected to warm desmolysis, in strong alkaline solution. Glycogen contained in desmolysis, in the presence of concentrated sulphuric acid, at warm, is divided in glucose which dehydrates and forms 5-hydroxymethylfurfural. Hydroxymethyl-5-furfural is condensing with antrona (9,10-dihydro-9-oxoantracen) resulting a compound coloured in green-blue,

colour intensity being direct proportional with glucose concentration which was obtained after hydrolysis of glycogen contained in the analysed tissue.

- Necessary reagents

1) Potassium hydroxide solution 30 %;

2) Sulphuric acid solution 95 %;

At 5 ml distilled water are added 100 ml sulphuric acid and it is chilled.

3) Antrona solution 0.2 % in sulphuric acid 95 %;

Dissolve 0.2 g antrona in 100 ml sulphuric acid 95 %. Reagent is extremely instable so this is the reason why will be prepare with at least 1 hour before determination, being utilised only in that day.

- Working way

Into a glass thermo-resistant sample tube with dimensions 20×150 mm are poured 3 ml potassium hydroxide solution (reagent 1) and closed with a rubber stopper. After that at analytical balance is weighted a quantity of 0.1 – 0.5 g of analysed tissue (liver, muscle), chopped in small pieces and will be introduced in the potassium hydroxide solution from glass tube. Stopper is removed and tube in placed for 20 minutes in water bath for boiling. After chilling with tap water, tube content is moved into a volumetric flask with a capacity of 50 - 100 ml, washing for many times with 4 - 5 ml of distilled water, complete till sign and shake strongly. From the obtained glycogen solution is measured a quantity of 0.5 – 2.5 ml into a thermo-resistant glass tube with dimensions of 30×200 mm and if it is necessary the volume will be completed up to 2.5 ml with distilled water. At the same time in another identical glass tube will be poured 2.5 ml of distilled water for control of reagents. Both tubes will be placed into a iced water bath. After chilling, in each tube is measured from a burette, with care, and into a continuous thin thread under a continuous shake 5 ml of antrona solution in sulphuric acid 95 % (reagent 3). Tubes will be covered with a glass pear and will be introduced for 10 minutes into a boiling water bath. After that will be chilled on a icy water bath and immediately will be read the extinction at spectrophotometer at wave length $\lambda = 620$ nm (Artenie, VI., Ungureanu E., Negură A., 2008).

- Calculation of results

In according with extinction value could be founded on etalon curve glycogen quantity (mg) from the studied solution volume. After that is calculated the glycogen content, expressed in mg %, in the studied tissue in according with the formula:

$$X = (a \cdot V_1 \cdot 100) / (V_2 \cdot P) \text{ mg \%}$$

in which:

a - glycogen quantity, in mg, founded on etalon curve;

V1 - volume (ml) at which was diluted the desmolysis obtained after treatment with potassium hydroxide;

V2 - volume of glycogen solution (ml) utilised for colour reaction;

P - mass of the analysed tissue (g);

100 - coefficient for transforming in percents.

- Design of etalon curve

To design the etalon curve is utilised a glycogen solution which is made diluting

exactly 40 mg of glycogen into a 500 ml volumetric flask in hot distilled water. After chilling of solution at room temperature, the volume is completed with distilled water till sign and it is shaking. This solution has 0.08 mg glycogen into a millilitre. From the obtained solution are prepared a series of samples for designing the etalon curve, pouring into thermo-resistant glass tubes with dimensions of 30×200 mm, solution volumes in according with the below presented table 1.

Is recommended that adding of antrona solution 0.2 % in sulphuric acid 95 % to be made at certain time intervals (for example 2 minutes) from one glass tube to the another in series. This time interval will be respected also at reading of extinction after chilling of samples at room temperature. Extinction of chilled samples will be read at a spectrophotometer with wave length of $\lambda=620 \text{ nm}$.

Table 1 Design of etalon curve

Reagent	Glass tubes								
	C	1	2	3	4	5	6	7	8
Glycogen quantity (mg)	0	0.02	0.04	0.06	0.08	0.10	0.12	0.16	0.2
Standard solution of glycogen (ml)	0	0.25	0.50	0.75	1.00	1.25	1.50	2.00	2.5
Distilled water (ml)	2.5	2.25	2.00	1.75	1.50	1.75	1.00	0.50	0
Samples are chilled on icy water bath									
Antrona solution (ml)	5	5	5	5	5	5	5	5	5
Samples will be placed for 10 minutes into a water bath for boiling and after that are chilled in tap water After chilling of glass tubes will read the extinction at $\lambda=620 \text{ nm}$									

RESULTS AND DISCUSSIONS

Research aimed to determine the glycogen content from muscular tissue and hepatic tissue of studied trout. At the end of effectuated analysis was calculated a mean of those 40 individuals from each experimental batch (table 2).

Obtained data after analysis effectuated of hepatic tissue recorded mean values of $2.325 \pm 0.892 \text{ g/100g}$ for rainbow trout and of $3.832 \pm 0.450 \text{ g/100g}$ for brook trout. In the case of muscular tissue were recorded mean values of $0.098 \pm 0.016 \text{ g/100g}$ for rainbow trout and of $0.130 \pm 0.044 \text{ g/100g}$ for brook trout.

Table 2 Glycogen quantity (expressed in g/100 g tissue) from hepatic and muscular tissue gathered from analysed individuals

Breed	Analysed tissue			
	Hepatic tissue		Muscular tissue	
	$\bar{x} \pm S_x$	V%	$\bar{x} \pm S_x$	V%
<i>Oncorhynchus mykiss</i>	2.325 ± 0.892	38.36	0.098 ± 0.016	16.67
<i>Salvelinus fontinalis</i>	3.832 ± 0.450	11.75	0.130 ± 0.044	33.93

CONCLUSIONS

Determination of glycogen content in different animal tissues has a relevant importance for investigation of physiological and pathological states of animal organism and also for research of influence of certain factors on carbohydrates metabolism.

Concluding we can say that from the point of hepatic glycogen and also of muscular glycogen, trout from Cheița trout farm have remarkable quantitative reserves, less exposed to variations, which enlightened well rearing and developing conditions.

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