THE TOTAL DNA QUANTIFICATION FOR THREE TYPES OF TISSUE FROM *CARASSIUS AURATUS GIBELIO* BLOCH

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Keywords: total DNA, *Carassius auratus gibelio* Bloch.

Abstract: We established the total DNA quantity and the variability intervals for three types of tissue (muscle, liver and spleen) from five individuals of *Carassius auratus gibelio* Bloch, to characterize this species from the point of view of this parameter.

MATERIALS AND METHODS

Biological material used for DNA extraction was represented by 5 individuals for each species which come from Larga Jijia - Movileni fishing farm. The establishment of DNA quantity was made after the extraction with phenol: chloroform: isoamylalcohol (25 : 24 : 1) (Ausubel 1995). The technique was used for DNA extraction from fresh tissues. For cellular lyses we used a lyses buffer and K proteinase which were incubated at 37°C for 12 hours. After the incubation period the samples were centrifuged 2 times, each time for separate the first layer from liquid column (figures 1 – 3).

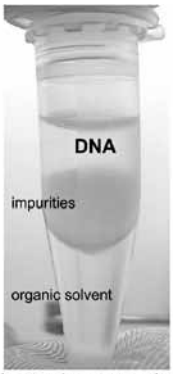

Fig. 1. First separation

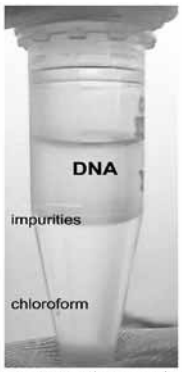

Fig. 2. Second separation

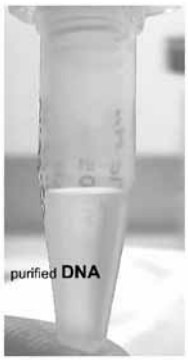

Fig. 3. Purified DNA

After the final separation, the purified DNA is precipitated in absolute ethanol kept at -20°C (figure 4) and centrifuged for pellet obtaining. All pellets were resuspended in TE buffer (pH=8.0) and kept at -20°C.

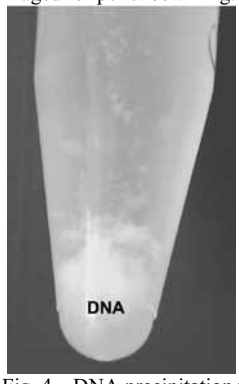

Fig. 4 – DNA precipitation with ethanol at -20°C

RESULTS AND DISSCUSIONS

Based on absorption values at 270nm and pure DNA extract, we created an etalon curve and a regression equation (figure 5). The regression equation was used to find the real values of DNA concentration from analysed tissues. For a better comparison between all samples, we represented the average values in a graph (figure 6).

For the establishment of total DNA variability intervals were calculated the standard error and the both limits (superior and inferior limits) for each interval. All variability intervals were represented in figure 7.

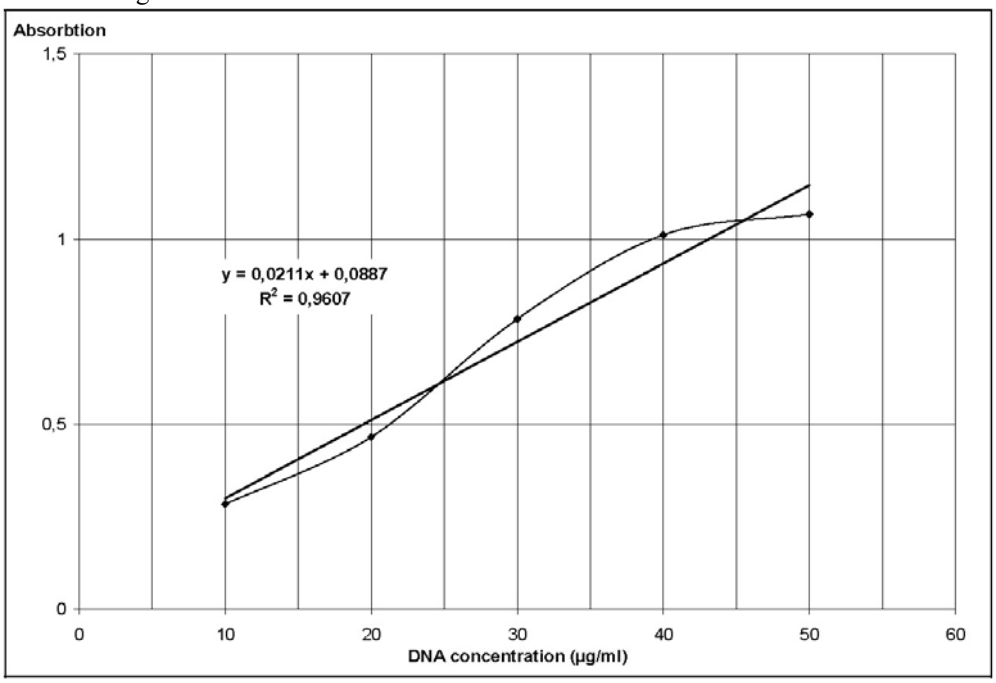

Fig. 5 DNA’s etalon curve, corresponding to λ=270nm absorption

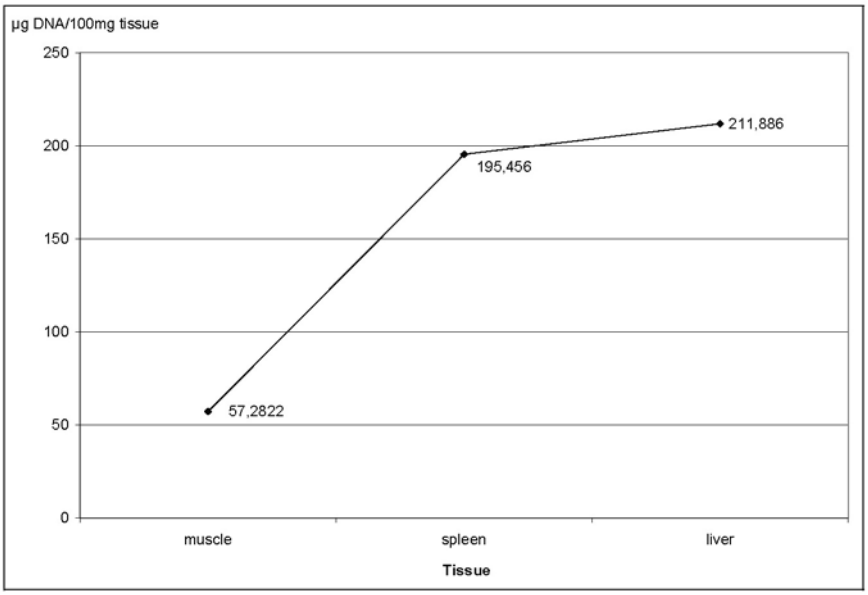

Fig. 6 Graphic presentations for average’s values

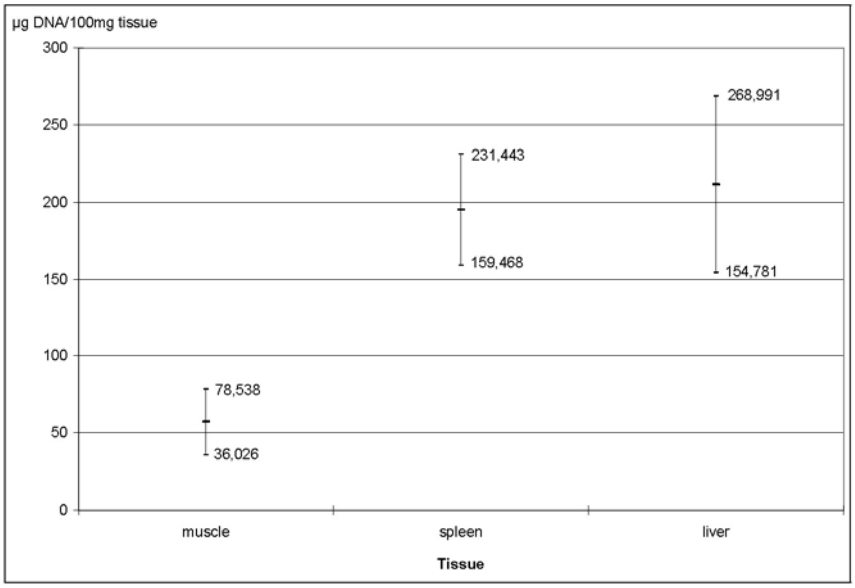

Fig. 7 Variability intervals

From variability intervals graphic representation, we observe that the intervals dimension grows directly proportional with the DNA quantity; for muscle tissue the limits vary between 36,026µg DNA/100mg tissue (for lower limit) and 78,538 µg DNA/100mg tissue (for higher limit), and for liver, vary between 154,781 µg DNA/100mg tissue (for lower limit) and 268,291 (for higher limit).

CONCLUSIONS

The smallest values are recorded for muscle tissue and the highest for liver tissue.

Referable to variability intervals we can observe that the interval dimensions growth proportional with DNA quantity.

REFERENCES

Ausubel, F. M., Brent, R., Kingston R. E., Moore D. D., Seidman J. G., Smith J. A., Struhl K., 1995– “*Current protocols in molecular biology*”, vol. 1, cap. 2 – Preparation and analysis of DNA. Phenol extraction and ethanol precipitation of DNA, Ed by John Wiley & Sons, Inc. 2.1.1. – 2.1.3.

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