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# COMPARISON OF GENETICALLY IMPROVED AND CONVENTIONAL RAINBOW TROUT LINES: PRELIMINARY RESULTS FROM CZECH AQUACULTURE

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## Abstract

Between 2022 and 2025, five rainbow trout lines of different origins were tested in aquaculture, including two lines with genetically selected resistance to pathogens. Morphometric traits, tissue composition, haematological parameters, and mortality were evaluated. The Czech line from Hynčice showed the best overall condition, a higher proportion of energy reserves, and stable growth throughout the year. The Italian lines exhibited stable growth but had the lowest immune indicators and the highest mortality. The Danish lines showed balanced performance; however, their growth was dependent on water temperature. Genetic resistance in the Danish improved lines was only weakly expressed under natural farming conditions, but during early rearing stages they exhibited lower mortality. The results highlight the importance of selecting an appropriate line with respect to local conditions and economic considerations.

Keywords: rainbow trout, RAS, QTL line, aquaculture

## INTRODUCTION

Aquaculture is currently the fastest-growing food production sector worldwide (Halim *et al.*, 2025) and plays an important role in global food security by providing 49 % of all aquatic products consumed (Choudhury *et al.*, 2023).

Within this context, rainbow trout (*Oncorhynchus mykiss*) culture represents an important part of global aquaculture, standing out with 1.72 % in 2020 (Papadopoulos *et al.*, 2024). Its high growth rate and nutritional content make it an attractive option for human consumption (Laiboud *et al.*, 2022). It is the dominant salmonid in Europe and North America, although its production faces challenges related to animal welfare, culture densities, and water quality (Roy *et al.*, 2021). In addition, it is exposed to diseases such as infectious pancreatic necrosis (Salgado-Miranda, 2006) and environmental risks such as heavy metal accumulation, excessive use of water resources, waste generation, and impacts on natural aquatic ecosystems (Martínez-Tabche *et al.*, 2002).

In response to these challenges, recirculating aquaculture systems (RAS) have emerged as a sustainable technological alternative for the culture of high-value aquatic species (Grandez-Yoplac *et al.*, 2025). RAS represent an intensive technology that allows the reuse of more than 95 % of water through mechanical and biological processes, such as biofiltration and disinfection (Jacinda, 2021). These systems significantly reduce water consumption and environmental impact (Rodríguez-Leal *et al.*, 2023), improving biosecurity, disease control, and efficiency in aquaculture production (Patrón *et al.*, 2024).

However, producers face technical, economical and legislative challenges such as high energy consumption, operating costs, and a restricted range of authorized therapeutic agents (Lindholm-Lehto, 2022). One potential approach is the use of trout lines with specific declared traits, such as resistance to common diseases. In the context of adaptation to climate change, acute hyperthermia and hypoxia resistance are also traits of growing interest in aquaculture. The feasibility of genetic improvement of these resistance traits through selection has been demonstrated in rainbow trout (Lagarde *et al.*, 2025). Such lines can be supplied directly by breeding institutions (e.g., Aquasearch Ova ApS – Denmark, Troutlodge – USA, AquaGen – Norway), or existing lines can be propagated by facilities responsible for their maintenance (e.g., Società Agricola Pescicoltura Burrini S.S. – Italy, Freia Forellen – Finland, Nordic Trout – Denmark, Farma Hate – Slovakia). Alternatively, facilities may use their own stock for spawning, either from original or previously acquired lines.

In this study, we took the opportunity to compare different rainbow trout lines directly under aquaculture conditions. The fish farm had begun searching for alternative lines to those previously used, primarily due to the susceptibility of the cultured lines to different infections. This allowed a direct comparison of their production performance under practical farming conditions, as well as selected morphological and hematological parameters related to fish physiology. Lines were tested in various experimental designs as well as in parallel in farming conditions, including assessment by the fish farmer. However, it is important to emphasize that the comparison of lines was limited. Since the testing was carried out directly at the fish farming facility in order to best reflect standard fish farming conditions, it was necessary to take into account, for example, the limited number of simultaneously reared lines, the number of fish used for analyses, or the need not to disrupt/respect the operation and conditions of the facility.

## MATERIALS AND METHODS

Fish rearing was conducted in a RAS at BioFish s.r.o., Pravíkov. The system had a total water volume of 1 000 m³, with the rearing section consisting of twelve parallel tanks, each 11 m long, 2 m wide, and 1.6 m water depth (≈35 m³ per tank). Water temperature ranged from 0.5 to 22 °C, with an average of approximately 10.5 °C.

In the farm, several lines of rainbow trout are usually raised simultaneously. Two triplets of lines (of different origins and declared traits) bred in parallel were monitored. Group A – one line of Czech origin (CZ) and two lines of Italian origin (IT-1, IT-2), group B–a CZ line and two lines of Danish origin (DA-0, DA-I) with declared traits, including resistance to selected diseases (Tab. I).

In 2023–2024, group A were monitored, while in 2024–2025 group B. The IT-1 and CZ lines were delivered in the early juvenile stage, whereas DA lines were acquired at the eyed-egg stage and subsequently reared under local conditions. The IT-2 originated from on-site spawning using broodstock of line IT-1 maintained at the facility. The experiment compared fish of the same age, beginning at the early juvenile stage. Fish were fed Biomar Inicio and Biomar ORBIT diets, with pellet sizes adjusted to match fish size. All lines were harvested upon reaching market size (~0.4 kg per fish). Fish were stocked in each tank at a density of approximately 5000 individuals per tank.

Measurements and determinations of selected parameters were conducted on the corresponding number of individuals (see below) to assess fish condition and physiology in both groups; the parameters were evaluated separately for each group. Parameters included body dimensions and weight (for calculation of condition indices), hematological indicators (erythrocyte and leukocyte counts, including differential counts, and phagocytic activity), gill surface area. Tissue composition analysis (dry matter) was done for group B only. Operational data from facility staff were also recorded concurrently.

For the assessment of condition-related parameters, 10 individuals from each line were evaluated. Fish size was measured (standard length, total length,

I: *Description of the monitored lines of rainbow trout, their designation and origin.*

| Group | Abbr. | Linie Name | Supplier | Line Description |
|---|---|---|---|---|
| A | IT-1 | | Societa'agricola pescicoltura burrini S.S | No genetic advantage, but appears tolerant to PKD |
| A | IT-2 | | Own Rearing | Derived from Italian line IT-1 |
| A, B | CZ | Hynčický pstruh | Hynčice | Reared at the Hynčice trout hatchery, Hradec Králové Region |
| B | DA-0 | Aquasearch FRESH | Aquasearch Ova ApS | No genetic advantage |
| B | DA-I | Aquasearch QTL RTFS + ICH | Aquasearch Ova ApS | Genetic advantage with combined resistance to flavobacteriosis and ichthyophthiriosis |

body height, body width) as well as body weight (total body weight, gutted weight, hepatopancreas weight and spleen weight). Based on these data, Fulton's condition factor ($Fc = W/SL^3$) and Clark's condition factor ($Cc = wt/SL^3$) were calculated as indicators of fish condition. Furthermore, the indices of body depth and body width ($Ih = SL/H$; $Iw = W/SL \times 100$), as well as the hepatosomatic, viscerosomatic, and splenosomatic indices ($HSI = wh/W$; $VSI = (W - w)/W$; $LSI = wl/W$), were determined.

Heparinized blood was used for the determination of erythrocyte and leukocyte counts (adaptive immunity), for the preparation of blood smears, and for the measurement of phagocytic activity (innate immunity). Blood was collected by puncture of the caudal vessels. Blood smears were prepared immediately after blood collection and were stained after air-drying (Hemacolor, Merck, Germany). Erythrocytes and leukocytes were counted manually using a Bürker counting chamber (Svobodová *et al.*, 2012). Differential leukocyte counts were determined from stained blood smears (Piačková *et al.*, 2013); the observed relative proportions of individual leukocyte types were recalculated to absolute numbers. Phagocytic activity was measured using a chemiluminescence assay (Papežíková *et al.*, 2016). The results were expressed as peak values and integrals of the kinetic curves. A total of 10 individuals per line were evaluated.

Gill surface area was expressed as the product of the number of primary lamellae and their height on the first left gill arch. Lamella height was calculated as the mean of ten lamellae located at equal intervals along the measured gill arch. For comparison, relative values were used, i.e. absolute gill surface area in relation to total body weight. A total of 10 individuals per line were examined.

The basic composition of fish tissues (whole body, muscle, organs) was analysed for dry matter, fat, ash, and nitrogenous substances. Dry matter content was determined from fresh samples as the percentage ratio of dried to fresh sample weight. Fat content was measured by Soxhlet extraction of pre-dried homogenized samples. Ash content was determined by incineration of a pre-dried homogenized sample and expressed as the ratio of ash weight to the weight of the pre-dried sample. Whole-body samples from four individuals per line (CZ, DA-0, DA-I) were analysed. Organ samples were pooled from five fish and evaluated as one representative sample per line. This approach as a standard procedure in situations where the amount of available tissue material is low and it is not possible to analyse each fish separately. For liver and visceral tissues, the amount of material is limited; therefore, a composite (pooled) sample from five individuals is prepared to obtain at least a group-level estimate. This procedure is in line with the recommendations issued by the United States Environmental Protection Agency.

Mortality of individual lines was assessed using cumulative mortality (percentage of individuals that died from stocking to a given time point) and monthly mortality (number of dead individuals per month). Fish mortality was recorded from routine farm logs.

### Statistical Analysis

All statistical analyses were undertaken using the Unistat for Excel statistical software package (version 6.5). As a first step, all datasets were assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Where both assumptions were met, differences between the means of the three groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons, while an unpaired t-test was applied for comparisons between two groups. If the data did not meet assumptions of normality and/or homogeneity of variance, differences between the three groups were assessed using Kruskal-Wallis ANOVA, with Dunn's test employed for post-hoc multiple comparisons, with the Mann-Whitney U test used for two-group comparisons. In all cases, statistical significance was set at $p < 0.05$.

## RESULTS AND DISCUSSION

### Meristic

The average total length and standard length of the monitored fish lines ranged from 209–259 mm and 179–227 mm, respectively, with body weights between 109 and 222 g (Tab. II).

The condition factor exhibited statistically significant higher values in the CZ line in both monitored groups, indicating a generally more robust body conformation. In comparison with the DA lines, statistically significant differences were also detected in the indices of body depth and body width. Lines of IT origin showed higher values of the hepatosomatic, viscerosomatic, and splenosomatic indices relative to the other lines, suggesting increased storage of energy reserves in the hepatopancreas and visceral adipose tissue. Comparable index values have also been reported by Mareš *et al.*, (2016), these observations were made in studies assessing the condition parameters of rainbow trout under different rearing technologies at Pravíkov. Compared to the CZ line, the DA and IT lines were generally slenderer. Overall, the CZ line exhibited superior condition and a more robust body conformation relative to the other lines, indicating a favourable genetic predisposition of this line for cultivation under local rearing conditions.

### Gills

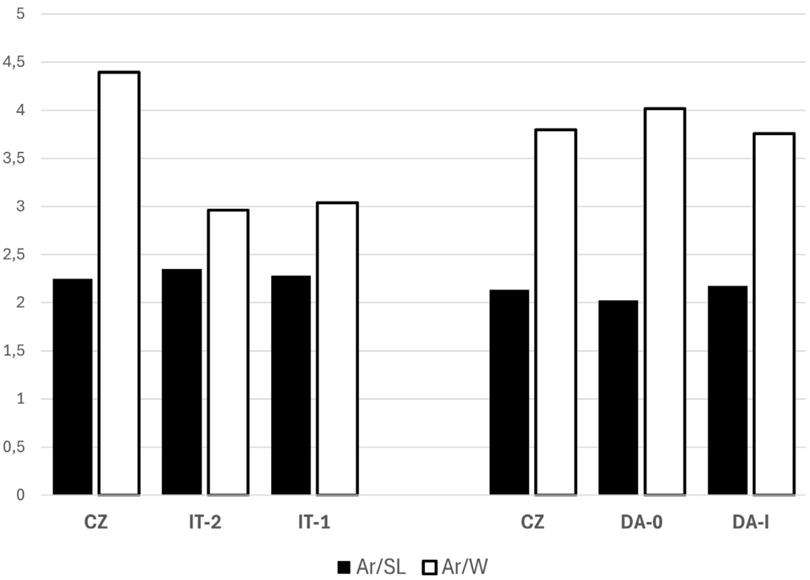
Since gill morphology, including the number of lamellae and their length, changes during the growth of an individual (e.g., Bigman *et al.*, 2023), absolute

II: *Morphological parameters of the monitored lines in group A and B. The table shows the average value of ten analysed individuals per line. Groups A and B were evaluated separately, within the respective monitored period. Significant differences between lines are indicated in bold and by different superscripts.*

| Parameter | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | CZ | IT-1 | IT-2 | CZ | DA-0 | DA-I |
| TL (mm) | **208.00 ± 11.30[a]** | **256.00 ± 14.1[b]** | **259.00 ± 20.00[b]** | 252.00 ± 8.70 | 258.00 ± 13.5. | 250.00 ± 10.80 |
| SL (mm) | **179.00 ± 11.60[a]** | **225.00 ± 15.00[b]** | **227.00 ± 19.60[b]** | 220.00 ± 6.58 | 227.00 ± 11.80 | 221.00 ± 10.90 |
| H (mm) | **46.70 ± 3.68[a]** | **57.60 ± 4.48[b]** | **60.70 ± 5.58[b]** | **60.60 ± 4.87[a]** | **53.10 ± 4.85[b]** | **53.30 ± 4.40[b]** |
| W (mm) | **23.60 ± 2.22[a]** | **30.40 ± 3.24[b]** | **30.30 ± 2.87[b]** | 28.60 ± 1.69 | 27.40 ± 2.20 | 27.40 ± 2.50 |
| $w_t$ (g) | **109.00 ± 17.90[a]** | **205.00 ± 39.50[b]** | **222.00 ± 51.00[b]** | 213.00 ± 24.8 | 196.00 ± 38.30 | 189.00 ± 28.70 |
| $w_v$ (g) | **93.00 ± 14.70[a]** | **173.00 ± 33.40[b]** | **184.00 ± 43.60[b]** | 180.00 ± 20.40 | 170.00 ± 34.00 | 163.00 ± 26.60 |
| $w_h$ (g) | **0.99 ± 0.25[a]** | **2.85 ± 0.52[b]** | **3.14 ± 0.74[b]** | 2.28 ± 0.36 | 2.08 ± 0.44 | 1.96 ± 0.56 |
| $w_s$ (g) | **0.15 ± 0.05[a]** | **0.31 ± 0.06[b]** | **0.33 ± 0.12[b]** | 0.23 ± 0.07 | 0.19 ± 0.04 | 0.21 ± 0.05 |
| $F_C$ | 1.87 ± 0.13 | 1.77 ± 0.13 | 1.89 ± 0.21 | **2.02 ± 0.09[a]** | **1.67 ± 0.10[b]** | **1.75 ± 0.10[b]** |
| $C_C$ | 1.61 ± 0.12 | 1.50 ± 0.09 | 1.56 ± 0.16 | **1.70 ± 0.07[a]** | **1.44 ± 0.10[b]** | **1.50 ± 0.09[b]** |
| HSI | **1.04 ± 0.12[a]** | **1.66 ± 0.20[b]** | **1.72 ± 0.25[b]** | 1.26 ± 0.09 | 1.24 ± 0.21 | 1.23 ± 0.42 |
| VSI | 14.00 ± 1.85 | 15.30 ± 1.52 | 17.10 ± 2.18 | 15.70 ± 0.90 | 13.60 ± 2.38 | 14.00 ± 1.58 |
| SSI | 0.16 ± 0.05 | 0.19 ± 0.06 | 0.18 ± 0.08 | 0.13 ± 0.03 | 0.12 ± 0.04 | 0.13 ± 0.04 |
| IW | 13.20 ± 0.88 | 13.50 ± 0.81 | 13.40 ± 1.10 | **13.10 ± 0.49[a]** | **12.10 ± 0.62[a,b]** | **12.40 ± 0.85[b]** |
| IH | 3.85 ± 0.21 | 3.92 ± 0.26 | 3.75 ± 0.29 | **3.63 ± 0.22[a]** | **4.28 ± 0.20[b]** | **4.16 ± 0.25[b]** |

TL – total length, SL – standard length, H – body height, W – body width, wt – total weight, wv – weight without viscera, wh – hepatopankreas weight, ws – spleen weight, Fc – Fulton index, Cc – Clark index, HSI – hepatosomatic index, VSI – viscerosomatic index, SSI – splenosomatic index, IW – wideback index, IH – widhtback index

values must be normalized to the size or weight of the individual when making comparisons, which may allow for a different interpretation of the results obtained. In our study, we used total body weight, which is expected to better reflect the metabolic requirements of the organism than size measurements and revealed more pronounced differences compared to using fish size alone. The relative gill area (i.e., the height and number of primary lamellae on the first gill arch normalized to body weight) was lower in the IT lines (Fig. 1, Tab. III) compared to the other lines. Considering




1: *Differences in relative gill surface area in relation to body weight and size in both groups*
Ar – relative area gill arch, SL – standard length, W – weight

III: *Gill morphology in groups A and B. Groups were evaluated separately, within the respective monitored period. Significant differences are indicated in bold and by different superscripts.*

| Parameter | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | CZ | IT-1 | IT-2 | CZ | DA-0 | DA-I |
| **W (g)** | **93.40 ± 14.70[a]** | **173.00 ± 33.40[b]** | **187.00 ± 45.30[b]** | **111.00 ± 15.80[a]** | **118.00 ± 17.00[a]** | **95.30 ± 13.10[b]** |
| Nl | 81.50 ± 7.26 | 85.20 ± 5.31 | 86.30 ± 4.33 | 86.90 ± 4.26 | 82.80 ± 5.61 | 82.30 ± 2.06 |
| **Hl (mm)** | **4.96 ± 0.34[a]** | **5.76 ± 4.48[b]** | **6.18 ± 0.79[b]** | **4.77 ± 0.19[a]** | **5.28 ± 0.70[b]** | **4.58 ± 0.14[a]** |
| **Aa ($mm^2$)** | **403.00 ± 32.70[a]** | **513.00 ± 45.20[b]** | **534.00 ± 76.60[b]** | **415.00 ± 26.40[a]** | **438.00 ± 68.80[b]** | **377.00 ± 13.20[a]** |
| **Ar (Aa/W)** | **4.39 ± 0.58[a]** | **3.04 ± 0.46[b]** | **2.96 ± 0.58[b]** | 3.80 ± 0.44 | 3.76 ± 0.66 | 4.02 ± 0.58 |

W – weight, Nl – number of primary lamellas, Hl – average height of primary lamellas, Aa – absolute area gill arch, Ar – relative area gill arch

that, in addition to respiration, fish gills are the primary sites for osmoregulation, excretion of nitrogenous waste products, and metabolism of hormones and xenobiotics (Evans *et al*., 2005), this finding can be regarded as a potential disadvantage. A genetically determined lower tolerance to hypoxia, caused by a reduced gill area, has been reported in triploid rainbow trout by Barahona *et al*. (2025). The importance of gills is also recognized in ecotoxicological studies, where relevant morphological parameters, such as the volume of the primary gill lamellae, are routinely monitored (Fiedler *et al*., 2020).

## Hematology

### *Erythrocytes*

Within both monitored groups, the CZ line exhibited higher erythrocyte counts and hemoglobin content, although these differences were not statistically significant (Tab. IV). Statistically significant differences among the lines were also not found in hemoglobin concentration or hemoglobin content per erythrocyte. A notable finding was the smaller erythrocyte volume observed in the CZ line. The potential trend of higher erythrocyte counts combined with smaller cell size in the CZ line may be considered a possible advantage, as smaller erythrocytes can, for example, pass more easily through the narrow capillary networks of the circulatory system. This may contribute to more efficient blood flow and help maintain or increase oxygen transport capacity, which could be adaptive for the organism, particularly during higher physical activity or under more demanding environmental conditions.

The range of basic hematological parameters in erythrocytes (ERY 0.8–1.5; HCT 0.24–0.55; HB 76–160; MCHC 0.14–0.26; MCH 55–82; MCV 276–476) in rainbow trout, as reported by Mocanu *et al.* (2013), demonstrates a relatively wide variability, within which the results obtained in our study generally fall.

Changes in hematological parameters can be induced, for example, by infections (Zorriuhzahra *et al*., 2010) or, conversely, by therapeutic interventions (Saglam *et al*., 2003; application of malachite green). The influence of feed composition enriched with antioxidants (Besharat *et al*., 2024; nanoliposome-coated astaxanthin), probiotics (Mocanu *et al*., 2013; *Bacillus licheniformis* and *B. subtilis*), or plant extracts (Farahi *et al*., 2010; *Allium sativum*) has also been demonstrated, leading to increases in practically all of the above parameters, in some cases by approximately 10 %. The increased values observed in the IT lines compared to the CZ line suggest that a similar effect may also be achieved using appropriate genetic variants in cultured fish.

IV: *Erythrocyte parameters in groups A and B. The table shows the average value of ten analysed individuals per line. Groups were evaluated separately, within the respective monitored period. There were no significant differences between lines.*

| | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | CZ | IT-1 | IT-2 | CZ | DA-0 | DA-I |
| **ERY ($T.l^{-1}$)** | 1.06 ± 0.13 | 0.99 ± 0.16 | 0.96 ± 0.15 | 0.90 ± 0.08 | 0.86 ± 0.14 | 0.82 ± 0.14 |
| **HK ($l.l^{-1}$)** | 0.34 ± 0.03 | 0.34 ± 0.03 | 0.34 ± 0.03 | 0.29 ± 0.03 | 0.31 ± 0.02 | 0.29 ± 0.03 |
| **HB ($g.l^{-1}$)** | 86.20 ± 9.87 | 82.40 ± 6.63 | 81.30 ± 13.30 | 70.30 ± 4.24 | 69.30 ± 5.14 | 64.30 ± 5.19 |
| **MCHC ($l.l^{-1}$)** | 0.26 ± 0.01 | 0.24 ± 0.01 | 0.24 ± 0.02 | 0.24 ± 0.01 | 0.22 ± 0.02 | 0.23 ±0.01 |
| **MCH (pg)** | 81.20 ± 5.05 | 84.60 ± 10.60 | 85.60 ± 15.70 | 78.50 ± 5.53 | 82.40 ± 11.40 | 80.90 ± 16.20 |
| **MCV (fL)** | 317.00 ± 23.00 | 351.00 ± 50.10 | 362.00 ± 55.60 | 329.00 ± 27.40 | 369.00 ± 46.20 | 360.00 ± 69.20 |

ERY – erythrocyte count, HK – hematocrit, HB – hemoglobin concentration, MCHC – mean corpuscular hemoglobin concentration, MCH – mean corpuscular hemoglobin, MCV – mean corpuscular volume

### *Leukocytes*

In both groups, the CZ line exhibited a significantly higher leukocyte count compared to the two IT lines, predominantly represented by lymphocytes (Tab. V). Lymphocytes are the primary cells of adaptive immunity, responsible for mounting a specific immune response against pathogens, including viruses and bacteria. The IT-1 line, however, had higher numbers of granulocytes and neutrophils compared to CZ, which are part of the innate immune system responsible for rapid nonspecific protection and pathogen phagocytosis. This may reflect an acute infection. CZ shows increased leukocytes in both groups (A and B), suggesting that this is more likely a stable characteristic (possibly a genetic predisposition to higher lymphocyte counts) rather than just a random, short-term response.

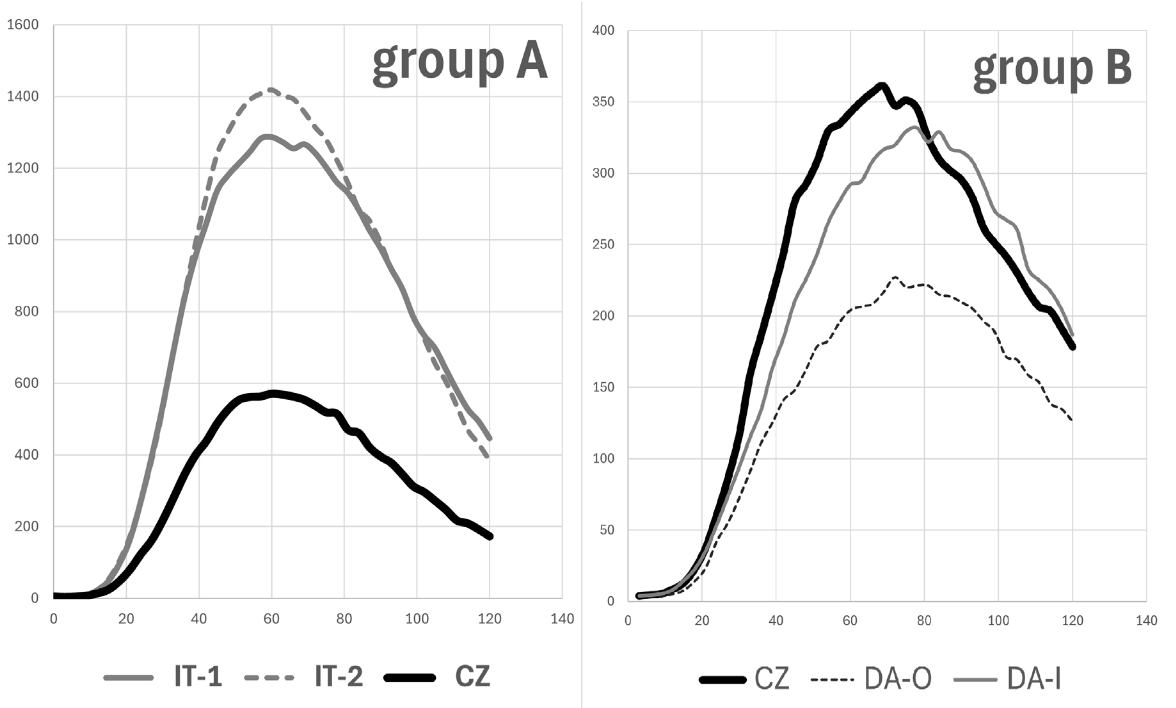
### *Phagocytic Activity*

Within group A, significant differences in phagocytic activity were observed—the integral and peak values were significantly lower in the CZ line compared to the other two groups (Fig. 2). However, when the values were recalculated per 1,000 phagocytes in the sample, no significant differences between the groups were detected. The peak-time value, reflecting the speed of onset of the phagocyte response to the activator, did not differ significantly among the groups.

No significant differences in phagocytic activity were detected among the individual lines within group B—neither in the intensity of the response (peak and integral) nor in the speed of its onset (peak-time). However, significant differences were found in phagocytic activity recalculated per

V: *Leukocyte parameters in groups A and B. The table shows the average value of ten analysed individuals per line. Groups were evaluated separately, within the respective monitored period. Significant differences are indicated in bold and by different superscripts.*

| | Group A | | | Group B | | |
|---|---|---|---|---|---|---|
| | CZ | IT-1 | IT-2 | CZ | DA-0 | DA-I |
| Leucocyte (G.l$^{-1}$) | **20.60 ± 5.47$^{a}$** | **12.60 ± 3.39$^{b}$** | **12.20 ± 3.34$^{b}$** | 11.10 ± 3.70 | 8.80 ± 2.79 | 10.60 ± 2.79 |
| Lymphocyte (G.l$^{-1}$) | **19.90 ± 5.51$^{a}$** | **11.40 ± 3.35$^{b}$** | **11.30 ± 3.39$^{b}$** | 10.10 ± 3.45 | 8.27 ± 2.64 | 10.00 ± 2.74 |
| Granulocyte (G.l$^{-1}$) | 0.58 ± 0.34 | 0.98 ± 0.58 | 0.66 ± 0.55 | 0.88 ± 1.03 | 0.38 ± 0.50 | 0.37 ± 0.28 |
| Neutrophile (G.l$^{-1}$) | 0.05 ± 0.12 | 0.09 ± 0.11 | 0.05 ± 0.10 | 0.03 ± 0.05 | 0.01 ± 0.02 | 0.00 ± 0.00 |
| Monocyte (G.l$^{-1}$) | 0.05 ± 0.08 | 0.09 ± 0.10 | 0.15 ± 0.14 | 0.40 ± 0.43 | 0.14 ± 0.21 | 0.15 ± 0.22 |
| Fagocyte (G.l$^{-1}$) | 0.68 ± 0.44 | 1.16 ± 0.63 | 0.86 ± 0.69 | 1.17 ± 1.45 | 0.53 ± 0.67 | 0.52 ± 0.32 |




2: *Phagocyte activity of monitored lines. Each curve represents the average phagocytic activity of ten analysed individuals in each group.*

1,000 phagocytes in the sample. For the integral, a statistically significant difference was observed between the DA-0 and DA-I lines. For the peak, a statistically significant difference was detected between the DA-I line and both other lines.

### Tissue Composition

The most pronounced differences in body composition were observed in dry matter and fat content (Tab. VI). The whole body of fish from the CZ line showed a higher proportion of both dry matter and fat than fish from both DA lines, indicating greater energy reserves. Fish from the IT line were not evaluated, as they were no longer supplied to the system during the period when these parameters were measured. In muscle tissue, dry matter and fat values were higher in the CZ line than in the imported lines; however, these differences were not statistically significant. Differences were also observed in organs, particularly in the viscera, where the CZ line contained substantially more fat (over 50%) compared to the DA lines (approximately 33%).

Differences in the hepatopancreas were less pronounced; however, fat content was also higher in the CZ line than in the D-0 group. Statistical analysis was not performed for these samples because it was based on only one representative sample per group; nevertheless, the numerical values clearly indicate a consistent trend. Ash (mineral) content did not differ significantly among the groups, suggesting stable tissue mineralization. Nitrogenous substances (proteins) in the muscle tissue showed only minor differences and remained relatively stable, indicating that the proportion of the protein component is not markedly influenced by the line. The tissue composition of rainbow trout from the CZ line indicates a higher content of energy reserves in both the body and organs, which was reflected in better condition and a more robust body conformation. In the case of muscle tissue, values close to those obtained in our study were also reported by Mareš *et al.* (2017) for rainbow trout from the same fish-farming facility, based on stocks reared between 2012 and 2016, where the possible effect of two different feed mixtures was compared. The observed proportions of dry matter, protein, and fat in muscle tissue also correspond to the values reported for rainbow trout by Stoyanova *et al.* (2016) in fish reared in raceways and cages at the Forest Group Ltd fish farm in Bulgaria (dry matter 22.4 and 25.5%; protein 17.6 and 18.8 %; fat 3.6 and 5.2 %, respectively).

### *Mortality*

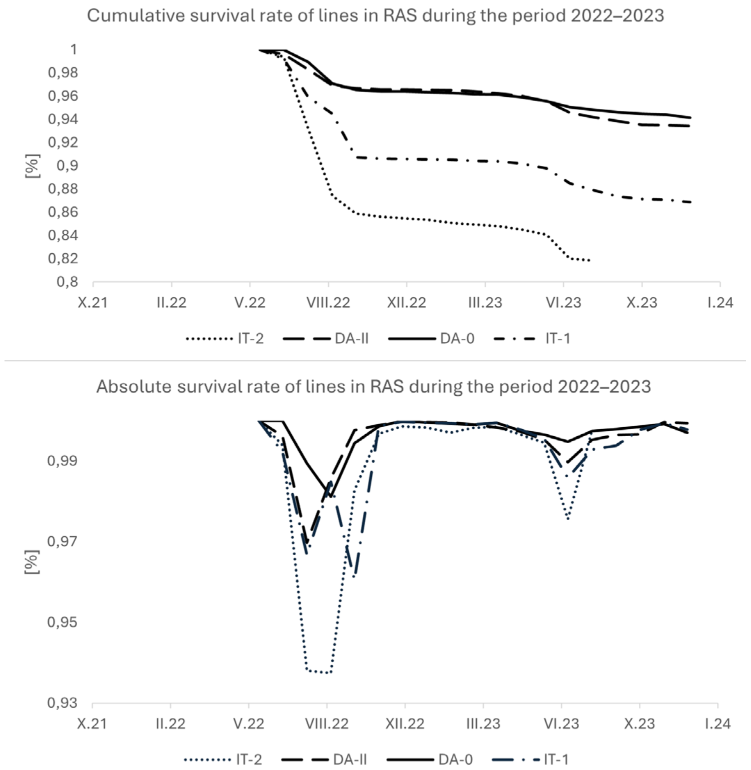
Additionally, the DA-II line with resistance to Flavobacterium was included in the mortality comparison. In the first monitored period between 2022–2023, lines IT-1, IT-2, DA-0, and DA-II were present in the RAS. As shown in Fig. 3, the highest mortality occurred in both IT lines, particularly in IT-2, this was confirmed by facility staff. A pronounced one-time drop in individuals (visible as peaks in the graph) was recorded during the summer of 2022, at the time of a severe outbreak of *Ichthyophthirius multifiliis* in the system. The difference in mortality between the IT and DA lines was approximately 6 %. These mortality patterns are consistent with hematological findings, as the IT lines exhibited the poorest adaptive immune function. Lines DA-0 and DA-II showed similar.

Another comparison was done in 2024–2025 using lines reared at the same time corresponding to group B. As in the first comparison, the DA lines did not differ significantly from each other in mortality.

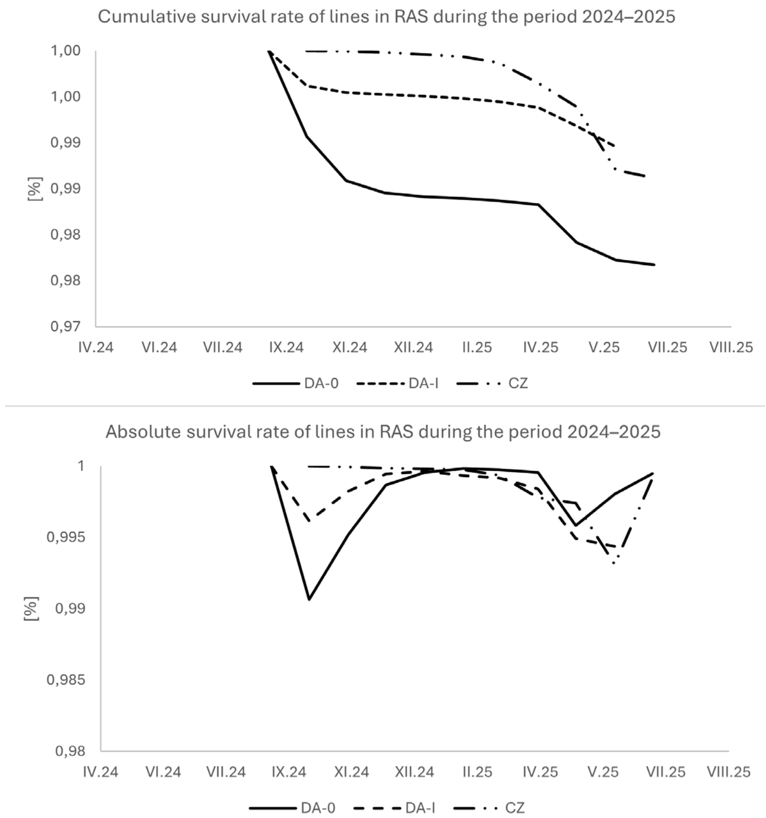
VI: *Tissue composition of rainbow trout in group B.*

| Parameter | | Group B | | |
|---|---|---|---|---|
| | | CZ | DA-0 | DA-1 |
| Dry matter (%) | [1] Whole body | **32.90 ± 0.60[a]** | **29.60 ± 0.93[b]** | **31.50 ± 0.15[c]** |
| | [1] Meat | 27.50 ± 1.97 | 26.50 ± 2.97 | 24.80 ± 1.58 |
| | [2] Viscera | 56.60 | 48.40 | 50.00 |
| | [2] Liver | 24.50 | 23.50 | 23.00 |
| Fat (%) | [1] Whole body | **15.50 ± 0.63[a]** | **11.20 ± 1.01[b]** | **13.50 ± 0.68[c]** |
| | [1] Meat | 6.40 ± 0.83 | 5.71 ± 1.35 | 5.60 ± 1.29 |
| | [2] Viscera | 52.70 | 33.00 | 33.40 |
| | [2] Liver | 3.53 | 3.23 | 3.76 |
| Ash (%) | [1] Whole body | 2.47 ± 0.67 | 2.65 ± 0.16 | 2.14 ± 0.10 |
| | [1] Meat | 1.54 ± 0.11 | 1.71 ± 0.24 | 1.50 ± 0.12 |
| Protein (%) | [1] Whole body | 15.70 ± 0.58 | 15.90 ± 0.50 | 15.80 ± 0.42 |
| | [1] Meat | 19.40 ± 1.77 | 19.30 ± 1.60 | 17.70 ± 0.39 |

[1] n = 4, [2] = composite sample, significant differences are indicated by different superscripts




3: *Cumulative (A) and absolute (B) survival curves in the RAS for lines IT-1, IT-2, and DA-0, DA-II between years 2022–2023. Each curve represents one line. Decline of the curves in summer 2022, indicating infection Ichthyophthirius multifiliis in the system.*




4: *Cumulative (A) and absolute (B) survival curves in the RAS for the DA-0, DA-I, and CZ lines between years 2024–2025. Each curve represents one line. In autumn 2024 and spring 2025 bacterial and Ichthyophthirius infections were present.*

Line DA-I performed slightly better than DA-0. Then CZ performed just as well, showing overall mortality slightly lower than DA-I. However, this difference may be due to initial differences in delivery. CZ line was purchased at the juvenile stage, whereas the DA lines are delivered as eyed eggs and then reared under local conditions. In autumn 2024 and spring 2025, infections with Ichthyophthirius and bacteria occurred, visible in Fig. 4 as peaks in the graph. In autumn, the genetic resistance of line DA-I likely manifested, as mortality was lower compared to DA-0. For the CZ line, the lower mortality may be attributed to the later stage of acquisition and the fact that it is purchased as juveniles.

### *Operational Information*

For the newly tested lines from Denmark, production staff were asked about their practical experience during rearing. All DA lines are imported at the eyed-egg stage (the IT and CZ lines are imported only at the juvenile stage) in very good condition, with minimal losses during hatching and after hatching. The hatched individuals are vital, with a very low percentage of deformed specimens. Initial feeding and the subsequent first growth phase (up to several grams) were completely trouble-free.

The DA lines showed the best growth during the spring and autumn periods, when water temperatures reached their optimal range of 7–15 °C. During the remaining parts of the year (winter and summer), they accepted less feed and their growth was slower compared to lines reared concurrently. Conversely, in the final growth phase, the DA lines showed a greater tendency toward increased size growth compared to the others. The CZ line exhibited stable growth throughout the entire year. In both DA lines, the fish did not exhibit (or only to a very minimal extent) gonadal development even at market size (up to 0.55 kg), and no breeder-undesirable behaviour changes associated with reproduction were observed (e.g. reduced feeding activity during the spawning period).

## CONCLUSION

The aim of our study was to provide a first insight and comparison of different types of lines, including those with declared genetic advantages, directly under practical farming conditions This approach, however, has its own limitations, including the possible occurrence of unexpected diseases, a relatively small sample size for analysis, and other uncontrolled factors that may influence the results. Therefore, these are preliminary results that we would like to further build upon in future research. At the same time, we tried to maximize the objectivity of the comparison by balancing the experimental groups in terms of fish age, rearing them in the same RAS, and exposing them to identical environmental conditions. This helped minimize the influence of other factors, such as differences in water sources, previous pathogen exposure, or variation in early developmental stages, which can affect mortality independently of genetic background.

In intensive fish farming, where rainbow trout often dominate, especially in RAS, the selection of suitable strains or lines of farmed fish is a key factor. Testing of different lines at the BioFish s.r.o. fish farm in Pravíkov revealed certain inter-line differences, concerning both core production parameters (mortality, body conformation) as well as additional parameters (haematology, chemical body composition). Overall tissue composition indicated a more robust body structure and better condition in the CZ line. The evaluated haematological parameters, including indicators of innate immunity (phagocytic activity) and adaptive immunity (white blood cell analysis), showed that genetic line has a significant influence particularly on the adaptive component of immunity. The IT lines exhibited significantly lower immune indicators compared to the CZ and consequently higher observed mortality in the rearing facility. Between the CZ line and the DA lines, statistically significant differences were found only for DA-0. Good adaptive immunity is further confirmed by the low mortality of the CZ and DA lines compared to the IT lines.

Under the local conditions of the Vysočina region, apart from the IT line, all other tested lines appeared similar, and no significant difference in survival/mortality was detected among QTL lines in RAS, except for the line with resistance to *I. multifiliis*. On the other hand, according to information from farm staff, DA lines with a genetic advantage showed better survival during the early rearing phase after hatching. The CZ and IT lines, in contrast, have the advantage of stable growth throughout the year, whereas DA lines exhibit stepwise growth only during periods of optimal temperatures for them. The selection of a line for a given production system is therefore a complex matter, and it is always necessary to test a line under specific local conditions.

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