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Original paper

Amino acid levels from blood plasma of rainbow trout raised in different seasons and farming systems

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Abstract

Climate change and large variations in medial parameters over the last decades, question the future of salmonid farming. For this, recirculating aquatic systems (RAS) have been developed, that artificially provide medial parameters at optimal values. To optimize these aquatic systems, fish are reared at high densities (50-100 kg / m^3 of water). From this point of view, due to high growth densities, the quality and biological value of trout meat can be depreciated. The results of our study show a relatively constant level of essential amino acids (EAA) and non-essential (NEAA) regardless of season for rainbow trout raised in RAS compared to those raised in the classic system. With small exceptions, both for EAA and NEAA, their values regardless of the season were higher in the case of RAS compared to the classic growth system. High level of valine (Val E Autumn 168.40 ± 1.14 μg / ml) is highlighted for the EAA compared with the level obtained in winter in the classical system (Val C Winter 105.40 ± 1.72 μg / ml). In NEAA, very high values were obtained for alanine (Ala E Autumn 117.60 ± 0.56 μg / ml) as compared to the same amino acid values in the winter season in the classical growth system (Ala C Winter 74.53 ± 0.67 μg / ml). The seasonal variations of EAA and NEAA in classical trout are due to environmental conditions, especially temperature, which influence the voluntary ingestion of feed, trout being poikilotherm organisms. Optimal media parameters in recirculating systems contribute to a higher biological value of trout, highlighted by the increased and constant level of EAA and NEAA.

Keywords *Salmonidae*, amino acids, metabolic activity, temperature, RAS.

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Introduction

Amino acids play an important role in fish metabolism (LI & al [1]), having direct implications in physiological processes such as digestion and enteral absorption, also serving as a substrate in protein synthesis (CARTER & al [2]; ANDERSEN & al [3]). Nutritional value of proteins is associated with amino acid content (FRIEDMAN [4]). This nutritional value is expressed by the intensity of growth, assimilation and immunity (LI & al [1]; BODIN & al [5]), respectively, in the defense capacity against biotic and / or abiotic stressors. Supplementation of some amino acids in the feed administered decreases the plasma cortisol levels (LEPAGE & al [6]) and they activate T and B lymphocytes, and antibody production (LI & al [7]). In general, fish's nutritional requirements towards amino acids do not differ greatly, most often due to age, environmental conditions, species (AKIYAMA & al [8]; MOHANTY & al [9]) and feeding behaviour (carnivorous, omnivorous, herbivorous, detritivorous) (WILSON & HALVER [10]). Being poikilotherm organisms, fish exhibit changes in behavior and some physiological parameters, depending on the season (COCAN & al [11]; MIREȘAN & al [12]), water temperature being one of the abiotic factors with major influence on the development of metabolic processes. Rainbow trout (*Oncorhynchus mykiss*), like other *Salmonidae* species, does not hibernate in winter as is the case of most fish species. However, it is difficult to withstand low water temperatures and the growth rate is reduced because it uses proteins for energy purposes. In this case, the constitutive amino acids are used in the synthesis of lipids (MARTINEZ & al [13]). In such situations it is recommended to increase the lipid level in feed (WEATHERUP & al [14]), because lipids are favoring protein assimilation (BILGUVEN & AK [15]).

Being a stenothermal species, rainbow trout has the thermal comfort limits ranging from 16-18ºC. In this thermal range, the metabolic processes are carried out with maximum intensity, being expressed by the dynamic growth. Exceeding this thermal range, as is more often in the case of summer season, due to climatic change and global warming (FROST & al [16]; MELERY & al [17]), it drives to dramatic issues involving a lowering of the voluntary intake rate and to the total refusal of the administered feed. In cases of hypoxia, caused by high temperature, there was a decrease in the concentration of amino acids involved in gluconeogenesis processes (alanine and aspartic acid), suggesting the tendency of metabolic activity to produce energy, to the detriment of anabolism (MÉDALE & al [18]; MARANDEL & al [19]). Based on selection, it is attempted to obtain rainbow trout lines with high tolerance for high temperatures (INENO & al [20]; CHEN & al [21]), which will no longer use protein resources for energy purposes.

Behaviorally, rainbow trout falls into the category of predatory species, which is why it requires a protein-rich but energetically balanced diet (MARANDEL & al [19]) respectively lipid and carbohydrate intake. Sometimes, in order to reduce production costs, vegetable protein substitutes (BURR & al [22]) are used, but in these cases artificial amino acid content is added (GAYLORD & BARROWS [23]) as dietary defects lead to a poor growth dynamic and a lower quality of fish meat. These aspects will be reflected in the economic losses of rainbow trout farmers.

To eliminate the problem of environmental variations that negatively affect the growth dynamics of rainbow trout and other fish species, recirculating aquatic systems (RAS) are highly used. In these systems, the medial parameters are artificially maintained at optimal values and in accordance with the biological requirements of the biological material. If these growth systems are effective in terms of growth dynamics, the question is "what is the quality of the final products obtained?". Similarly to other intensive farming systems (poultry, swine, cattle), in RAS the growth density is high (50-100 kg trout / m^3 of water). The discomfort caused by high growth density can lead to the occurrence of reactive oxygen species (peroxide, hydroxyl, superoxide) and to the installation of oxidative stress. It is manifested by the negative physiological effects, including a disorder of the metabolism and of the intestinal absorption. In this way, the quality of trout meat can be affected in terms of nutrients, including amino acids.

Knowing the correlation between circulating amino acids and meat constituents, the purpose of this study was to evaluate the level of the blood plasma amino acids of rainbow trout from two different growth systems, one classical (subject to environmental variation) and one recirculating (which does not depend on environmental variation), in the four seasons of the year.

Materials and Methods

1. Experimental design and biological material

The experiments were carried out in the period 2014-2015, in two locations: a classic system of rainbow trout breeding – Fiad trout farm, Bistrita-Nasaud county, respectively an intensive recirculating system, experimentally arranged in Cluj-Napoca, Cluj County. Each consisted of 500 specimens of rainbow trout. The genetic origin of the specimens taken into study was common, these coming from the nursery of the Fiad trout farm. The feeds used were the same for both lots (Aller ON Top Floating with a protein intake of 43% and a 22% lipid intake), and feed was made at the discretion of using self-service feeding systems. Trouts in both groups were female. The only variables were the environmental conditions for each growth system.

2. Collection and processing of blood samples

Each season, blood samples were collected from 25 trout specimens from each farming system. Three mililiters of blood was collected by cardiac puncture with syringes. The harvested samples were quickly transferred to 4 ml BD Li-Heparin vacuteiner tubes. Immediately after harvest, the samples were centrifuged at 4500 rpm for 5 minutes. The resulting blood plasma was transferred to eppendorf tubes with a volume of 2 mililiters, and the samples were then transferred to the laboratory for analysis in refrigerated containers at + 2°C.

3. GC-MC analysis of amino acids and working protocols

In order to evaluate the seasonal effects on rainbow trout amino acid concentration, we used the isotopic-mass spectrometric gas chromatography technique (Fig. 1, 2). The stable isotope used as the internal standard was ^{15}N labeled methionine.

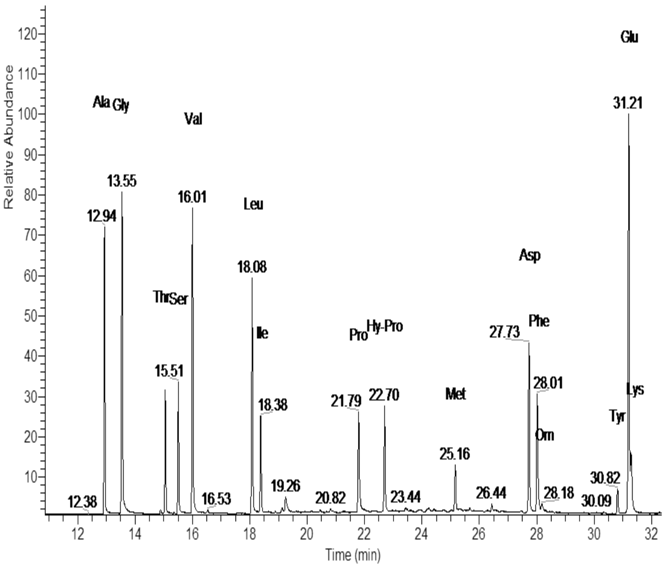

Figure 1. Chromatographic separation of blood plasma derivatised from amino acids

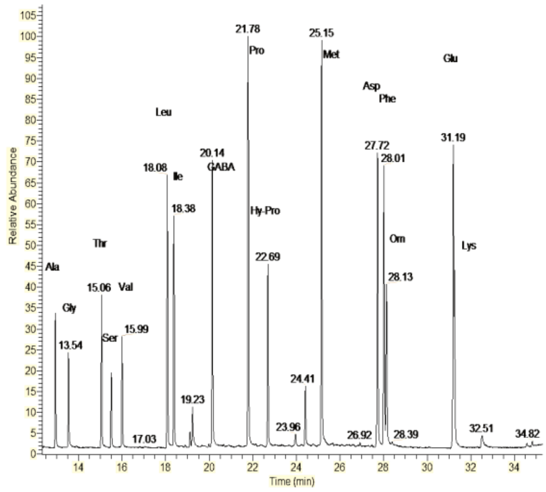

Figure 2. Chromatographic separation (standards)

A Mass Spectrometer with a quadrupole detector, Trace DSQ Thermo Finnigan, coupled with a gas chromatograph, Trace GC, was used. Amino acids were separated on a RTX-5MS capillary column of the following dimensions: 30 m x 0.25 mm and the deposited film thickness – 0.25 μm, using the following temperature program: stationing at 50°C for 1 minute, raising the temperature at a rate of 6°C / min. up to 100°C, with 4°C / min. up to 200°C and 20°C / min. up to 300ºC, where it stays for 3 minutes. The transfer line temperature was 250°C, the injector temperature was 200°C and the ion source 250°C. The set splitter ratio was 10: 1. The electron energy was 70 eV and the emission current was 100 μA. The amino acids were purified on a Dowex 50W-W8 ion exchange resin, subsequently derivatized by a two-step procedure, resulting in derivated trifluoroacetyl-butyl esters of amino acids. The amino acid identification was performed by looking at the NIST Spectrum Library, and with standard amino acids.

4. Statistical interpretation

For statistical interpretation of the data, GraphPad Prism 6.0.1 was used. With this program, we have calculated the mean values, standard deviation and standard error of the mean values, and differences between mean values by performing an ANOVA One-Way variance analysis. For awarding the differences between mean values of essential and nonessential amino acids, depending on the season and farming system, Tukey Multiple Comparison test was used, respectively “t” test (between farming systems).

Results and Discussion

A number of 21 amino acids have been identified and analyzed, of which 9 are essential, Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylanine (Phe), Threonine (Thr), Tryptophan (Trp) and Valine (Val) and 12 non-essential amino acids Alanine (Ala), Arginine (Arg), Aspartic acid (Asp), Glutamic acid (Glu), Alanine (beta-Ala), Cysteine (Cys), Glycine (Gly), Hydroxyproline (Hy-Pro), Ornithine (Orn), Proline (Pro), Serine (Ser) and Tyrosine (Tyr). The group farmed in the classical system (Fiad trout farm) is the control group (M), and the group in the recirculating aquatic system is the experimental one (E). It can be observed, in the case of the essential amino acids (Fig. 3) that between the two farming systems and regardless of the season, in most cases, higher values were assigned to the experimental group (E). The only close values, as expected, were recorded in the spring and autumn seasons, given that during these periods the external environmental conditions of the M-group were close to the biological requirements of the rainbow trout and in the recirculating system are artificially maintained conditions, regardless of the season. The insignificant differences were obtained with histidine in the spring season (d = 0.5759 μg / ml) and for lysine (d = 0.8026 μg / ml), methionine (d = 0.5130 μg / ml) and threonine = 0.4615 μg / ml) in autumn season. In most cases, especially in the winter season, the concentration of essential amino acids was higher in E group, resulting in very and extremely significant negative differences. Positive differences, favorable to the M group, were reported mainly in the spring and autumn seasons, with exceptions being recorded in the summer season, when lysine showed a higher value in the M group (103.70 ± 1.127 μg / ml) vs. E group (61.40 ± 1.104 μg / ml), resulting in an extremely significant difference of 42.27 μg / ml. Another amino acid that showed higher values in the M group during the summer season was phenylalanine (53.86 ± 0.845 μg / ml) vs. (33.59 ± 0.688 μg / ml) for E group, resulting in an extremely significant and positive difference (d = 20.27 μg / ml).

Similarly to the results obtained for EAA, in the case of NEAA, very and / or extreme significant differences were obtained between the two groups. It is again remarked that in the winter season, all the differences recorded were extremely significant and in favor of the group E (Fig. 4). In the autumn season, when the external environmental conditions are close to the biological and physiological requirements of rainbow trout for M group, it results in the case of several nonessential acids, in insignificant differences between groups.

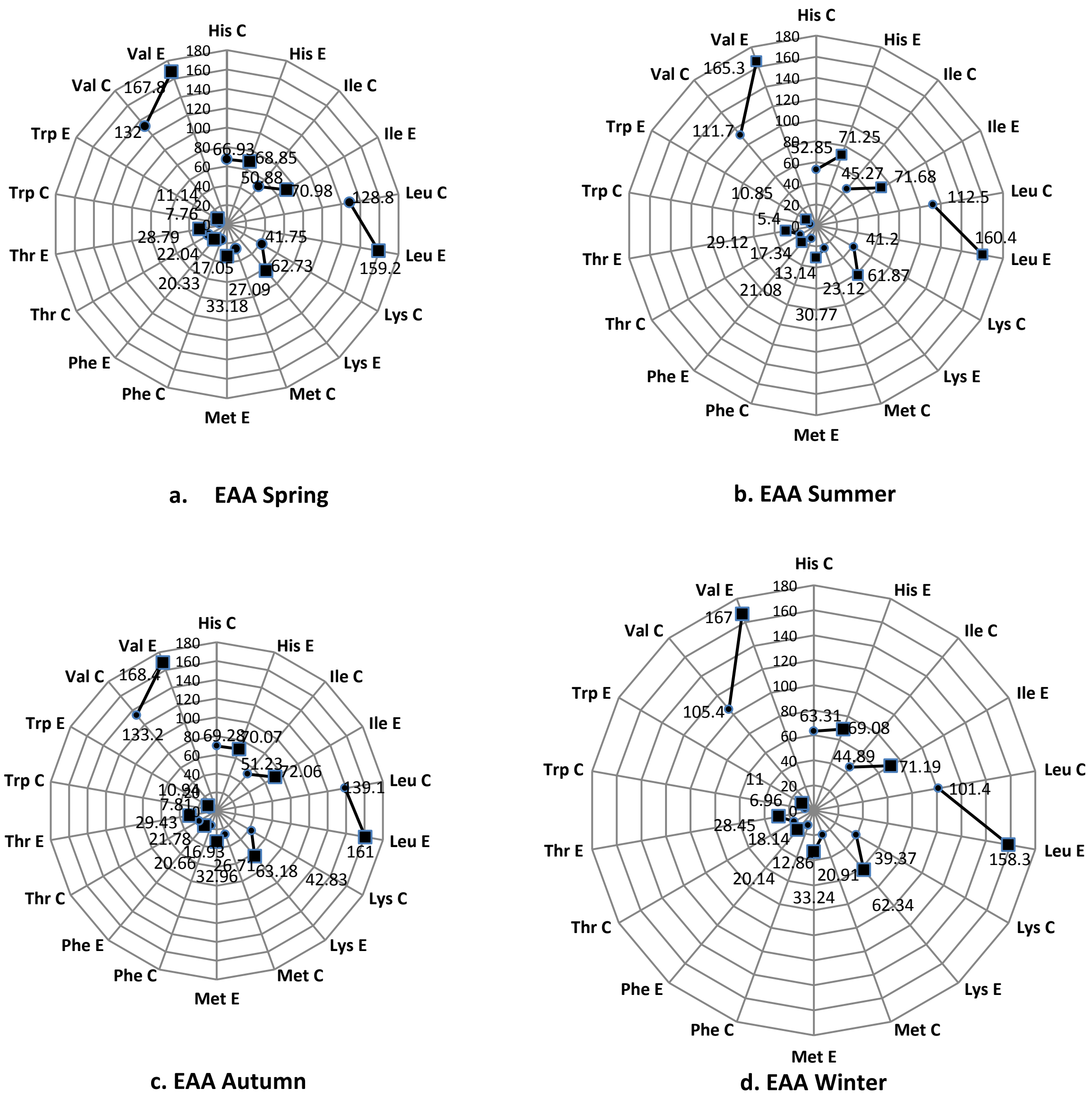

Figure 3. Mean values of essential amino acids (EAA- μg/ml) concentrations for the two groups (C-Control Group, E-Experimental Group)-seasonal values: a-Spring; b-Summer; c-Autumn; d-Winter

Thus, for arginine, values of 41.28 ± 0.835 µg / ml (M group) and 41.41 ± 0.630 µg / ml (E group) were obtained, resulting in an insignificant difference of 0.1329 µg / ml. Insignificant differences in amino acid concentration were also obtained for beta-Alanine (d = 1.174 µg / ml), proline (d = 1.853 µg / ml) and serine (d = 2.421 µg / ml).

But during the winter season, all the differences obtained were extremely significant and in favor of E group. During the summer season, the climatic conditions in Romania are not favorable to salmonid farming in a conventional system because of the increased water temperature. It is noticed a random distribution of differences, in the case of some amino acids (alanine, arginine, beta-alanine, cysteine, glycine and serine) in favor of E group and for the other amino acids (aspartic acid, glutamic acid, hydroxyproline, ornithine, proline and tyrosine), the differences being recorded in favor of M group (Table 1 and 2).

The circulating level of essential and non-essential amino acids in fish is mainly influenced by the quality of the administered feed (YUN & al [24]; YUN & al [25]) and the voluntary intake rate. Fish are poikilothermic organisms and any changes in medial parameters lead to behavioral and physiological changes (SARAVANAN & al [26]; HOLT & JØRGENSEN [27]). When medial parameters are in accordance with the biological requirements of the species, in this case, in the rainbow trout, metabolic rate with all its components (ingestion, digestive transit, secretory and mechanical digestive tract, enteral absorption, assimilation) is adequate. During the four temperate-continental climate seasons in which Romania is found, the temperature and dissolved oxygen values, as well as other physico-chemical parameters of the water, undergo normal and gradual variations.

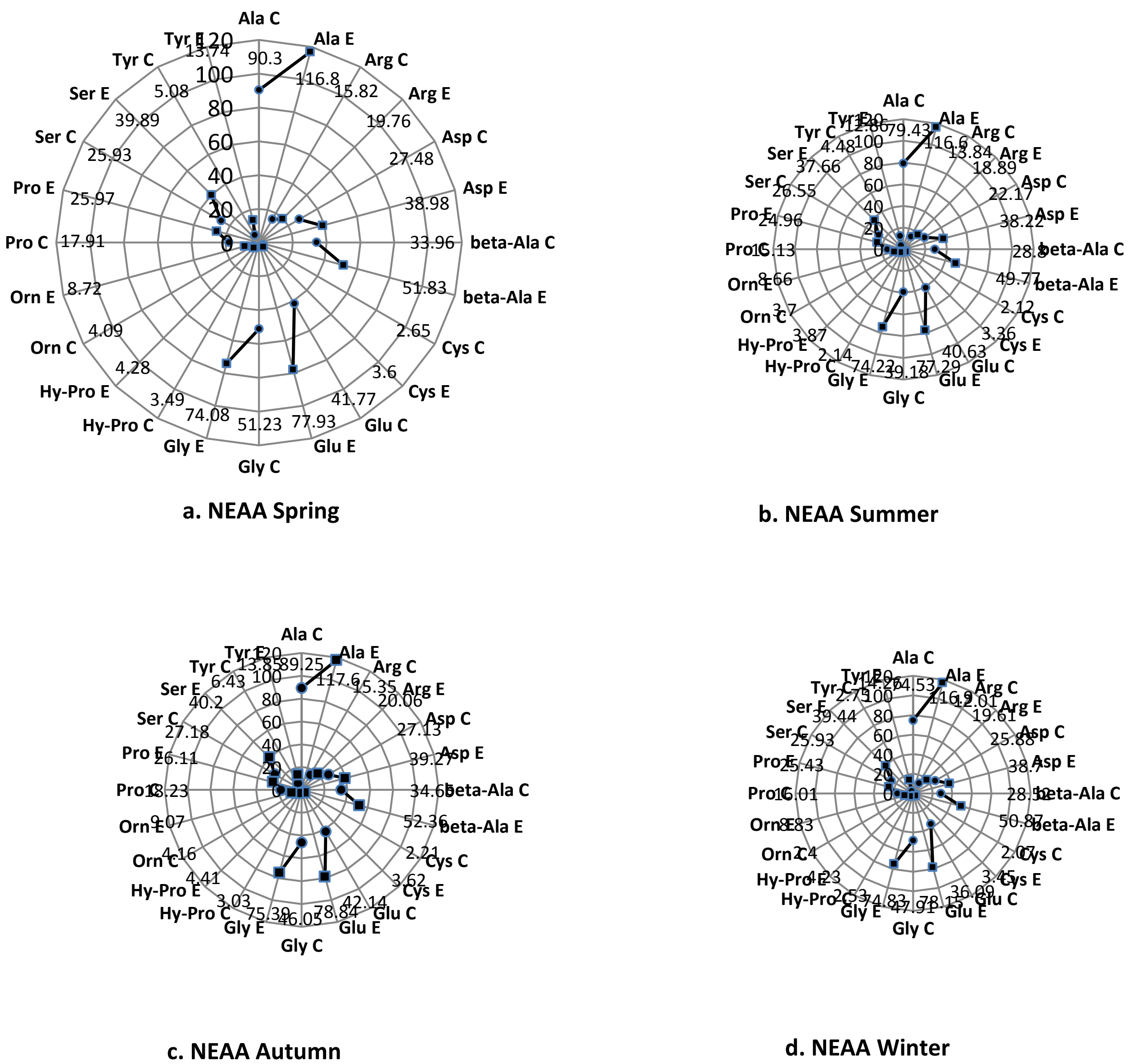

Figure 4. Mean values of non-essential amino acids (NEAA- µg/ml)concentrations for the two groups (C-Control Group, E-Experimental Group) – Seasonal values: a-Spring; b-Summer; c-Autumn; d-Winter

Table 1. Inter-seasonal analysis of variance (ANOVA One-Way) regarding the plasmatic concentration of essentials amino acids from rainbow trout (*Oncorhynchus mykiss*) blood plasma

EAA	Group	F	Spring-Summer	Spring-Autumn	Spring-Winter	Summer-Autumn	Summer-Winter	Autumn-Winter
His	C	150.90****	14.08****	2.35^{o}	3.62***	16.43^{oooo}	10.46^{oooo}	5.97****
	E	2.55^{ns}	2.40^{ns}	1.22^{ns}	0.23^{ns}	1.18^{ns}	2.17^{ns}	0.99^{ns}
Ile	C	33.49****	5.61****	0.35^{ns}	5.99****	5.96^{oooo}	0.38^{ns}	6.34****
	E	0.46^{ns}	0.70^{ns}	1.08^{ns}	0.21^{ns}	0.38^{ns}	0.49^{ns}	0.87^{ns}
Leu	C	309.50****	16.35****	10.31^{oooo}	27.42****	26.66^{oooo}	11.07****	37.73****
	E	1.17^{ns}	1.22^{ns}	1.88^{ns}	0.85^{ns}	0.66^{ns}	2.07^{ns}	2.73^{ns}
Lys	C	6.09***	0.55^{ns}	1.08^{ns}	2.38*	1.63^{ns}	1.83^{ns}	3.46***
	E	1.06^{ns}	0.86^{ns}	0.45^{ns}	0.39^{ns}	1.31^{ns}	0.47^{ns}	0.84^{ns}
Met	C	62.36****	3.97****	0.38^{ns}	6.18****	3.59^{oooo}	2.21***	5.80****
	E	5.36**	2.41**	0.22^{ns}	0.06^{ns}	2.19^{o}	2.47^{oo}	0.28^{ns}
Phe	C	121.20****	3.91****	0.12^{ns}	4.19****	3.79^{oooo}	0.28^{ns}	4.07****
	E	2.85*	0.75^{ns}	0.33^{ns}	0.19^{ns}	0.42^{ns}	0.94*	0.52^{ns}
Thr	C	156.60****	4.70****	0.26^{ns}	3.90****	4.44^{oooo}	0.80^{o}	3.64****
	E	1.04^{ns}	0.33^{ns}	0.64^{ns}	0.34^{ns}	0.31^{ns}	0.67^{ns}	0.98^{ns}
Trp	C	66.98****	2.36****	0.05^{ns}	0.80***	2.41^{oooo}	1.56^{oooo}	0.85***
	E	0.62^{ns}	0.29^{ns}	0.20^{ns}	0.14^{ns}	0.09^{ns}	0.15^{ns}	0.06^{ns}
Val	C	189.60****	20.32****	1.13^{ns}	26.69****	21.45^{oooo}	6.37***	27.82****
	E	1.13^{ns}	2.55^{ns}	0.57^{ns}	0.86^{ns}	3.12^{ns}	1.67^{ns}	1.43^{ns}

Note: ns – insignificant difference ($p>0.05$); * – significant positive difference ($p<0.05$); ** – distinctly significant positive difference ($p<0.01$); *** – very significant positive difference ($p<0.001$); **** – extremely significant positive difference ($p<0.0001$); ^{o} – significant negative difference ($p<0.05$); ^{oo} – distinctly significant negative difference ($p<0.01$); ^{ooo} – very significant negative difference ($p<0.001$); ^{oooo} – extremely significant positive difference ($p<0.0001$);

Table 2. Inter-seasonal analysis of variance (ANOVA One-Way) regarding the plasmatic concentration of non essentials amino acids from rainbow trout (*Oncorhynchus mykiss*) blood plasma

NEAA	Group	F	Spring-Summer	Spring-Autumn	Spring-Winter	Summer-Autumn	Summer-Winter	Autumn-Winter
Ala	C	111.80****	10.87****	1.05^{ns}	15.77****	9.82^{oooo}	4.90****	14.72****
	E	0.27^{ns}	0.28^{ns}	0.79^{ns}	0.09^{ns}	1.07^{ns}	0.37^{ns}	0.70^{ns}
Arg	C	58.19****	1.98****	0.47^{ns}	3.81****	1.51^{oooo}	1.83****	3.34****
	E	2.20^{ns}	0.87^{ns}	0.30^{ns}	0.16^{ns}	1.17^{ns}	0.72^{ns}	0.45^{ns}
Asp	C	78.43****	5.31****	0.35^{ns}	1.60***	4.96^{oooo}	3.71^{oooo}	1.25**
	E	0.41^{ns}	0.76^{ns}	0.29^{ns}	0.28^{ns}	1.05^{ns}	0.48^{ns}	0.57^{ns}
beta-Ala	C	33.22****	5.16****	0.69^{ns}	5.44****	5.85^{oooo}	0.28^{ns}	6.13****
	E	1.45^{ns}	2.06^{ns}	0.53^{ns}	0.96^{ns}	2.59^{ns}	1.10^{ns}	1.49^{ns}
Cys	C	34.86****	0.53****	0.44****	0.58****	0.09^{ns}	0.05^{ns}	0.14^{ns}
	E	2.40^{ns}	0.24^{ns}	0.02^{ns}	0.15^{ns}	0.26^{ns}	0.09^{ns}	0.17^{ns}
Glu	C	21.79****	1.14^{ns}	0.37^{ns}	5.68****	1.51^{ns}	4.54****	6.05****
	E	0.63^{ns}	0.64^{ns}	0.91^{ns}	0.22^{ns}	1.55^{ns}	0.86^{ns}	0.69^{ns}
Gly	C	98.81****	12.05****	5.18****	3.32****	6.87^{oooo}	8.73^{oooo}	1.86^{ns}
	E	0.41^{ns}	0.14^{ns}	1.31^{ns}	0.74^{ns}	1.17^{ns}	0.61^{ns}	0.56^{ns}
Hy-Pro	C	17.34****	1.35****	0.46****	0.96****	0.89^{oooo}	0.39^{oooo}	0.50****
	E	11.20****	0.41***	0.13^{ns}	0.05^{ns}	0.54^{oooo}	0.36^{oo}	0.18^{ns}
Orn	C	265.20****	0.39****	0.07^{ns}	1.69****	0.46^{oooo}	1.30****	1.76****
	E	2.84*	0.06^{ns}	0.35^{ns}	0.11^{ns}	0.41^{o}	0.17^{ns}	0.24^{ns}
Pro	C	55.00****	2.78****	0.32^{ns}	1.90****	3.10^{oooo}	0.88^{o}	2.22****
	E	1.13^{ns}	1.01^{ns}	0.14^{ns}	0.54^{ns}	1.15^{ns}	0.47^{ns}	0.68^{ns}
Ser	C	3.54*	0.62^{ns}	1.25^{ns}	0.54^{ns}	0.63^{ns}	1.16^{ns}	1.79*
	E	4.89**	2.23*	0.31^{ns}	0.45^{ns}	2.54^{oo}	1.78^{ns}	0.76^{ns}
Tyr	C	725.40****	0.60****	1.35^{oooo}	2.33****	1.95^{oooo}	1.73****	3.68****
	E	12.89****	0.88**	0.11^{ns}	0.52^{ns}	0.99^{ooo}	1.40^{oooo}	0.41^{ns}

Note: ns – insignificant difference ($p>0.05$); * – significant positive difference ($p<0.05$); ** – distinctly significant positive difference ($p<0.01$); *** – very significant positive difference ($p<0.001$); **** – extremely significant positive difference ($p<0.0001$); ^{o} – significant negative difference ($p<0.05$); ^{oo} – distinctly significant negative difference ($p<0.01$); ^{ooo} – very significant negative difference ($p<0.001$); ^{oooo} – extremely significant positive difference ($p<0.0001$);

As the physiological response of rainbow trout, when environmental factors are at extreme limits (generally in winter and summer), the intensity of metabolism is low. This aspect, with all its consequences, including a decrease in blood plasma of amino acid concentration and correlated with amino acid levels in meat, is normal. In the last decades, large and abnormal variations in climatic and meteorological parameters (CHITU & al [28]) are generally due to climate change (SANDERSFELD & al [29]). Although, there are evolutionary premises for adapting fish to climate change (NARUM & al [30]; McBRYAN & al [31]; HARDING & al [32]), in classical farming systems, trouts are subjected to acute stress as physiological adaptations to water parameters changing which takes time and are made gradually. When medial parameters change rapidly, a state of stress accurs due to the rapid response (LeBLANC & al [33]). The organism reacts by increasing the catecholamine levels (dopamine, epinephrine, norepinephrine, angiotensin), released into the blood in situations of physical or emotional stress (REID & al [34]; PERRY & BERNIER [35]; BARTON [36]). The high level of these similar hormones leads to the release of circulating glucose (GESTO & al [37]) and intensification of gluconeogenesis, while glycolysis is inhibited (WRIGHT & al [38]). In such cases, hyperglycemia is also an indicator of oxidative stress.

All of these physiological mechanisms and adaptations explain seasonal variations in the blood plasma amino acids of classical farmed trout (COCAN & al [11]). Hence, it can be concluded that due to the direct correlation between plasma and tissue (muscle) levels of amino acids, the quality and biological value of trout meat raised in the classical system is not constant. Similarly, the dynamics of fish growth is negatively influenced and therefore the technological flow is distorted, with no foresight on fish stocks being possible, thus affecting the economic component of aquaculture production.

Recirculating aquatic systems (RAS) originally emerged as an alternative to quantitative increase in yields. Nowadays, RAS offer optimal medial conditions to the biological material, which positively influences their qualitative components. The results of this study reflect the relatively constant level of essential and non-essential amino acids in the blood plasma of the rainbow trout farmed in RAS, regardless of the season.

Conclusions

The results of this study clearly highlight the importance of the medial parameters in terms of the quantitative and qualitative aspect of trout production. Climate change in recent decades is already a certainty, which is why new models and systems for trout farming are needed. Our research has demonstrated the beneficial influence of medial parameters (temperature, dissolved oxygen) provided in RAS on trout production and concentration in EAA and NEAA. We believe that such growth systems represent the future of salmoniculture both in Romania and globally. These systems are capable of producing high quality trout, with low water consumption.

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References

1. P. LI, K. MAI, J. TRUSHENSKI, G. WU. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37(1), 43, 53 (2009).
2. C. CARTER, S. OWEN., Z. HE, P. WATT, C. SCRIMQEOUR, D. HOULIHAN, M. RENNIE. Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss*, using a stable isotope. *Journal of Experimental Biology*, 189, 279, 284 (1994).
3. S.M. ANDERSEN, R. WAAGBØ, M. ESPE. Functional amino acids in fish nutrition, health and welfare. *Frontiers in Bioscience, Elite*, 8, 143, 169 (2016).
4. M. FRIEDMAN. Nutritional values of protein from different food sources. A review. *Journal of Agricultural and Food Chemistry*, 44(1), 6, 29 (1996).
5. N. BODIN, G. DELFOSSE, T.T.N. THU, E. LE BOULENGÉ, T. ABBOUDI, Y. LARONDELLE, X. ROLLIN. Effects of fish size and diet adaptation on growth performances and nitrogen utilization of rainbow trout (*Oncorhynchus mykiss* W.) juveniles given diets based on free and/or protein-bound amino acids. *Aquaculture*, 356-357, 105, 115 (2012).
6. O. LEPAGE, O. TOTTMAR, S. WINBERG, Elevated dietary intake of L-tryptophan counteracts the stress-induced elevation of plasma cortisol in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, 205, 3679, 3687 (2002).
7. P. LI, Y-L. YIN, D. LI, S.W. KIM, Aminoacids and immune function. *British Journal of Nutrition*, 98, 237, 252 (2007).
8. T. AKIYAMA, I. OOHARA, T. YAMAMOTO, Comparison of essential amino acid requirements with A/E ratio among fish species (Raview Paper). *Fisheries Science*, 63(6), 963, 970 (1997).
9. B. MOHANTY, A. MAHANTY, S. GANGULY, T.V. SANKAR, K. CHAKRABORTY, A. RANGASAMY, B. PAUL, D. SARMA, S. MATHEW, K.K. ASHA, B. BEHERA, M. AFTABUDDIN, D. DEBNATH, P. VIJAYAGOPAL, N. SRIDHAR, M.S. AKHTAR, N.SAHI, T. MITRA, S. BANERJEE, P. PARIA, D. DAS, P. DAS, K.K. VIJAYAN, P.T. LAXMANAN, A.P. SHARMA, Amino acid composition of 27 food

fishes and their importance in clinical nutrition. *Journal of Amino Acids*, Article ID 269797, 7 pages, 2014. doi:10.1155/2014/269797 (2014).

10. R.P. WILSON, J.E. HALVER. Protein and amino acid requirements of fishes. *Annual Review of Nutrition*, 6, 225, 244 (1986).
11. D. COCAN, E. HORJ, M. CULEA, V. MIREŞAN, A. PINTEA. Amino acids levels of rainbow trout (*Oncorhynchus mykiss*) plasma during spring-summer seasons. *Bulletin UASVM Animal Science and Biotechnologies*, 67(1-2), 132, 136 (2010).
12. V. MIREŞAN, D. COCAN, R. CONSTANTINESCU, C.RĂDUCU, O. NEGREA. Variation in blood parameters of rainbow trout (*Oncorhynchus mykiss*) under the influence of seasons and growth systems. *Bulletin UASVM Animal Science and Biotechnologies*, 71(2), 301, 302 (2014).
13. F.J. MARTINEZ, M.P. GARCIA, M. CANTERAS, J. De COSTA, S. ZAMORA. Simultaneous effect of initial weight, initial crowding, temperature and O2 concentration on the nutritional use of food by rainbow trout (*Oncorhynchus mykiss*). *Archives Internationales de Physiologie, de Biochimie et the Biophysique*, 100(3), 247, 250 (1992).
14. R.N. WEATHERUP, K.J. McCRACKEN, R. FOY, D. RICE, J. McKENDRY, R.J. MAIRS, R. HOEY. The effects of dietary fat content on performance and body composition of farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 151(1-4), 173, 184 (1997).
15. M. BILGUVEN, I. AK. The effects of different dietary protein and lipid levels and oil sources on the growth performance and body composition of rainbow trout (*Oncorhynchus mykiss*, W.). *European Scientific Journal*, 3(special edition), 807, 816 (2013).
16. A.J. FROST, J.S. THOMSON, C. SMITH, H.C. BURTON, B. DAVIS. Environmental change alters personality in the rainbow trout, *Oncorhynchus mykiss*. *Animal Behaviour*, 85(6), 1199, 1207 (2013).
17. J. MELLERY, F. GEAY, D.R. TOCHER, P. KESTEMONT, C. DEBIER, X. ROLLIN, Y. LARONDELLE. Temperature increase negatively affects the fatty acid bioconversion capacity of rainbow trout (*Oncorhynchus mykiss*) fed a linseed oil-based diet. *PLoS ONE*, 11(10), e0164478, (2016).
18. F. MÉDALE, J.P PARENT, F. VELLAS. Responses to prolonget hypoxia by rainbow trout (*Salmo gairdneri*) I. Free amino acids and proteins in plasma, liver and white muscle. *Fish Physiology and Biochemistry*, 3(4), 183, 189 (1987).
19. L. MARANDEL, I. SEILIEZ, V. VÉRON, S. SKIBA-CASSY, S. PANSERAT. New insight into the nutritional regulation of gluconeogenesis in carnivorous rainbow trout (*Oncorhynchus mykiss*): a gene duplication trail. *Physiological Genomics*, 47(7), 253, 263 (2015).
20. T. INENO, S. TSUCHIDA, M. KANDA, S. WATABE. Thermal tolerance of a rainbow trout *Oncorhynchus mykiss* strain selected by high-temperature breeding. *Fisheries Science*, 71(4), 767, 775 (2005).
21. Z. CHEN, M. SNOW., C.S. LAWRENCE, A.R. CHURCH, S.R. NARUM, R.H. DEVLIN, A.P. FARREL. Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Experimental Biology*, 218, 803, 812 (2015).
22. G.S. BURR, W.R. WOLTERS, F.T. BARROWS, R.W. HARDY. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). *Aquaculture*, 334-337, 110, 116 (2012).
23. T.G. GAYLORD, F.T. BARROWS. Multiple amino acid supplementation to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. *Aquaculture*, 287, 180, 184 (2009).
24. H. YUN, G. PARK, I. OK, K. KATYA, S. HUNG, S.C. BAI. Determination of the dietary lysine requirement by measuring plasma free lysine concentration in rainbow trout *Oncorhynchus mykiss* after dorsal aorta cannulation. *Fisheries and Aquatic Sciences*, 19(4), 1, 7 (2016).
25. H. YUN, G. PARK, I. OK, K. KATYA, S. HEUNG, S.C. BAI. Evaluation of optimum dietary threonine requirement by plasma free threonine and ammonia concentration in surgically modified rainbow trout, *Oncorhynchus mykiss*. *Asian-Australian Journal of Animal Sciences*, 28(4), 551, 558 (2015).
26. S. SRAVANAN, I. GEURDEN, A.C. FIGUEIREDO-SILVA, S. NUSANTORO, S. KAUSHIK, J. VERRETH, J.W. SCHRAMA. Oxygen consumption constrains food intake in fish fed diets varying in essential amino acid composition. *PLoS ONE* 8(8): e72757. doi:10.1371/journal.pone.0072757 (2013).
27. R.E HOLT, C. JØRGENSEN, Climate change in fish: effects of respiratory constrains on optimal life history and behaviour. *Biology Letters*, DOI: 10.1098/rsbl.2014.1032 (2015).
28. E. CHITU, D. GIOSANU, E. MATEESCU. The variability of seasonal and annual extreme temperature trends of the latest three decades in Romania. *Agriculture and Agricultural Science Procedia*, 6, 429, 437 (2015).
29. T. SANDERSFELD, F.C. MARK, R. KNUST. Temperature-dependent metabolism in Antarctic fish: Do habitat temperature condition affect thermal tolerance ranges? *Polar Biology*, 40(1), 141, 149 (2017).
30. S.R. NARUM, N.R. CAMPBELL, K.A. MEYER, M.R. MILLER, R.W. HARDY. Thermal adaptation and acclimation of ectotherms from differing aquatic climates. *Molecular Ecology*, 22(11), 3090, 3097 (2013).

31. T.L. McBRYAN, K. ANTTILA, T.M. HEALY, P.M. SCHULTE. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integrative and Comparative Biology*, 53(4), 648, 659 (2013).
32. M.M. HARDING, P.I. ANDERBERG, A.D.J. HAYMET. 'Antifreeze' glycoproteins from polar fish. *European Journal of Biochemistry*, 270, 1381, 1392 (2003).
33. S. LeBLANC, S. MIDDLETON, K.M. GILMOUR, S. CURRIE. Chronic social stress impairs thermal tolerance in the rainbow trout (*Oncorhynchus mykiss*). *The Journal of Experimental Biology*, 214, 1721, 1731 (2011).
34. S.G REID, N.J. BERNIER, S.F. PERRY. The andrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology*, 120(1), 1, 27 (1998).
35. S.F. PERRY, N.J. BERNIER. The acute humoral andrenergic stress response in fish: facts and fiction. *Aquaculture*, 177(1-4), 285, 295 (1999).
36. B.A. BARTON. Stress in fishes: a diversity of responses with particular references to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42(3), 517, 525 (2002).
37. M. GESTO, C. OTERO-RODIÑO, M.A. LÓPEZ-PATIÑO, J.M. MÍGUEZ, J.L. SOENGAS, M. CONDE-SIEIRA. Is plasma cortisol response to stress in rainbow trout regulated by catecholamine-induced hyperglycemia? *General and Comparative Endocrinology*, 205(1), 207, 217(2014).
38. P.A. WRIGHT, S.F PERRY, T.W. MOON. Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. *Journal of Experimental Biology*, 147, 169, 188 (1989).