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Differences in Nutritional Value and Amino Acid Composition of *Moina macrocopa* (Straus) Using Yeast *Saccharomyces cerevisiae* and *Rhodotorula glutinis* as Fodder Substrates

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Abstract: The nutritional composition and amino acid profile of *Moina macrocopa* were studied using different types of yeast (*Saccharomyces cerevisiae* and *Rhodotorula glutinis*) as fodder substrates. The effective accumulation of carotenoids in *Moina macrocopa* during *R. glutinis* yeast application was not accompanied by deterioration in the nutritional value of zooplankton. The content of total proteins and total lipids in *Moina* grown on *S. cerevisiae* and *R. glutinis* was not significantly different. However, the use of *R. glutinis* in the cultivation of *M. macrocopa* led to the change in the ratio of proteinogenic amino acids in the studied cladocerans. In particular, the share of methionine, leucine and isoleucine significantly increased. It allowed to enhance the quality of protein in the fodder zooplankton, that is especially important in the feeding of fish fry.

1. Introduction

The presence of carotenoids in feed is a prerequisite for ensuring high rates of fish growth and survival at different stages of development [1]. Like all animals, fish are not capable of synthesizing carotenoids, but can only transform them. Consequently, their admission into the fish body is conditioned of the feed assimilation. In addition, feed supplements enriched by xanthophylls, namely cantaxanthin and astaxanthin, are used to provide effective pigmentation of salmon fish meat [2]. Moreover, feeds enriched by carotenoids help to increase the organism's resistivity to various diseases, maximize fertility and inhibit mutagenesis. However, use of such supplements is often ineffective due to the low percentage of their ingestion by fish, as it is known that fish fry in the early stages of development prefers live feeds, namely zooplankton, in particular, representatives of crustaceans *Cladocera*.

As a source of carotenoids, various groups of microorganisms, including carotene-producing yeast, often attract attention. The expediency of their use as a source of carotenoids for a live feed is based not only on their high carotenosynthetic activity but also on their dimensional characteristics, which makes it possible for zooplankton to ingest them. The use of yeast as a fodder substrate for *M. macrocopa* is also appropriate due to the similarity of the amino acid composition of yeast and *Moina* [3].

It is important that the saturation of live feeds by some essential substances does not lead to a decrease in the content of other useful substances. Accordingly, the aim of our study was to find differences in the nutritional composition and amino acid profile of *M. macrocopa* as a live feed for fish fry while carotene-producing yeasts *R. glutinis* are used as a fodder substrate.

2. Materials and Methods

Cultivating conditions. The study was performed on the pure cultures of cladocerans *Moina macrocopa* (Straus, 1820) kept in the collection of the Institute of Biology, Chemistry and Bioresourses of Yuriy Fedkovych Chernivtsi National University (Chernivtsi, Ukraine). The cultivation of *Moina* was carried out in accordance with the generally accepted methodology [4]

using synthetic ADaM medium [5] in the jars of 0.5 l placed in the climatic chamber at a temperature of 21 ± 2°C, illuminated with 2500 lux fluorescent lamps for a 16-hour photoperiod. The initial number of the studied crustaceans was 50 individuals per liter.

Fodder substrate. Aqueous suspensions of the yeasts *Saccharomyces cerevisiae* Meyen ex E.C.Hansen (1883) and *Rhodotorula glutinis* (Fresenius) F.C. Harrison (1982) were used as a fodder substrate for the zooplankton. Yeast with the number of cells adjusted to 23.5–24.5×10^6 CFU per 1 liter of medium were applied every 48 hours. The yeast cells were counted using a Goryaev camera under a MicroMed XS-3300 binocular microscope. Preliminary studies have determined the optimal duration of yeast passage, which was 10 days, with 48 hours interval of introducing yeast suspension [6].

Yeasts were cultivated in a meat peptone broth. The inoculum was obtained by growing a seed culture for 2 days at 27°C. After that yeasts were cultivated in an LOIP LS-110 (160 rpm) laboratory shaker at 25–27°C for five days. The microbial suspension was centrifuged for 20 min. at 1500 g to separate the cultivated broth from the yeast biomass.

Biochemical analysis. The sampling for biochemical analysis was carried out in the phase of maximum productivity. The concentrated samples of zooplankton were treated with a USDN-2T ultrasonic desintegrator. Homogenization of the material with was carried out at +4°C in a Potter-Elvehjem homogenizer using phosphate buffer pH 7.4 with subsequent centrifugation of the homogenate at 1500 g for 15 min. The determination of total lipids extracted by Folch's method [7] was carried out by acid hydrolysis of the test samples, followed by the reaction between the decomposition products and phospho-vanillin reagent [8]. The total protein content was determined by Lowry's method [9]. The determination of amino acids content was performed by the method of ion-exchange liquid-column chromatography on T 339 automatic amino acid analyzer (Prague, Czech Republic). Amino acids in eluate were detected by the ninhydrin method [10]. The content of the individual amino acids was expressed as a percentage of total weight of amino acids. The determination of Trp amino acid was not performed. The determination of Asn and Gln content was realized together with Asp and Glu, respectively.

Studies on the total content of carotenoids were carried out according to the method [11]. Fractional composition of carotenoids was established by thin-layer chromatography (TLC) [12]. Identification of carotenoid fractions was conducted according to the identity of the absorption spectra in the range of 400–750 nm in the solvent (acetone) to the spectra of carotenoids given in the literature [13, 14].

All calculations were performed on dry weights, and to that effect the samples were dried at 105°C for 24 hours until they reached a constant weight [15].

Statistical analysis. The results were analyzed statistically with Microsoft Excel software and Single Factor ANOVA Tukey HSD test, STATISTICA 6.0 application package, according to generally accepted methods [16]. Mean values were considered significantly different at $P \leq 0.05$ according to Student's criterion.

3. Results and Discussion

Preferably microalgae are the natural feed for zooplankton. However, mainly *Saccharomyces cerevisiae* yeasts are used by fish farms during intensive cultivation of fodder zooplankton. Changing mineral and biochemical food qualities are common challenges to life history traits of zooplankters [17]. The results of our previous studies have shown that substitution of yeast *S. cerevisiae*, the traditional fodder substrate, by carotene-producing yeast *R. glutinis* in the cultivation of crustaceans *M. macrocopa* contributed to the accumulation of carotenoids up to 14 mg/g of dry weight, whereas in *Moina* individuals cultivated on *S. cerevisiae* the content of carotenoids did not exceed 0.1 mg/g. The use of *R. glutinis* does not reduce the dynamics of culture growth [6].

TLC technique identified 7 fractions of carotenoids in the composition of the total carotenoids in *M. macrocopa* using *R. glutinis* as the fodder substrate: astaxanthin and its monoesters, canthaxanthin, β-carotene, echinenone, ζ-carotene and lutein. The last 4 fractions account for only 0.21% of the total content of carotenoids (Fig. 1).

It should be noted that *R. glutinis* yeast itself accumulates carotenoids in the amount of 65 mg/g with the highest share of β-carotene (80%), in addition these yeasts contain torulene and torularhodin [18]. The emergence of carotenoids in *M. macrocopa*, that differ from the carotenoid fractions present in the yeast, apparently, caused by their transformation (metabolism) in the organism of cladocerans.

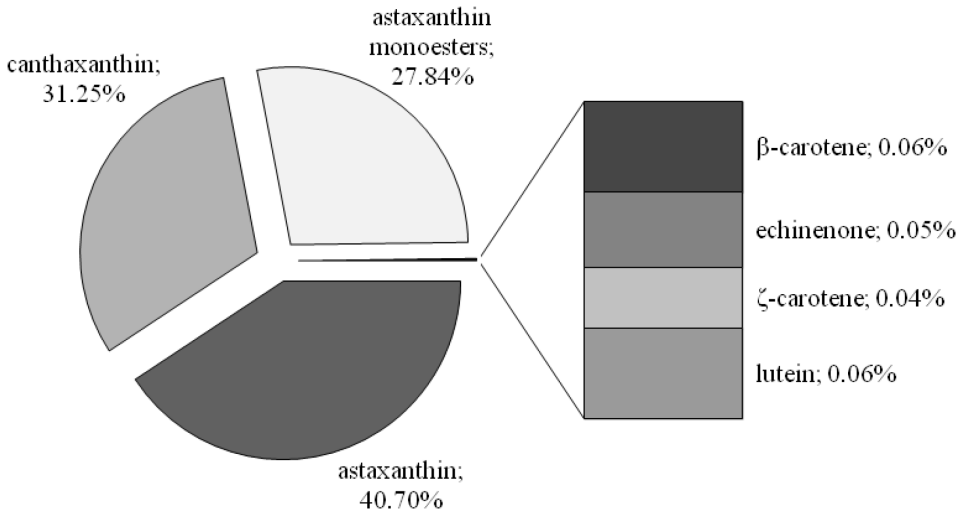

Figure 1. The mass share of individual carotenoid fractions in *Moina macrocopa* while using *Rhodotorula glutinis* yeast as a fodder substrate.

On the other hand, the question was whether the active storage of carotenoids in zooplankton did not lead to a redistribution of the ratio of basic nutrients and to a decrease in the nutritional value of live feed in general.

The obtained data showed that the content of total proteins and total lipids in *M. macrocopa* during its feeding with *R. glutinis* and *S. cerevisiae* was not significantly different (Table 1).

Table 1. The nutritional composition of *Moina macrocopa* while using *Saccharomyces cerevisiae* and *Rhodotorula glutinis* as a fodder substrate.

Fodder substrate	**Nutritional composition of cladocerans per dry weight**	
	Total proteins, *mg/g*	Total lipids, *mg/g*
S. cerevisiae	510.4±45.66	126.5±34.42
R. glutinis	555.1±24.08	115.9±8.21

An important characteristic of the feed is not only the protein content, but also the ratio of individual amino acids in the fodder protein. The use of protein with an unbalanced amino acid profile does not provide the expected rate of biomass increasing in hydrobionts due to the high energy consumption of the body for transamination processes, as well as increases the level of excess nitrogen intake to water, which creates an additional burden on water purification systems. In accordance with the listed above, recent studies, that develop the concept of ideal protein for certain species of hydrobionts, have gained popularity [19, 20].

Usually, when analyzing the amino acid composition of feed for fish, special attention is paid to the content of essential amino acids, insufficiency of which inhibits growth, reduces digestibility of feed and adversely affects the viability of fish [21]. Providing fodder organisms such as

M. macrocopa with essential amino acids in sufficient quantities, on the one hand, increases the nutritional value of these organisms as feed, and on the other, it helps to accelerate the increase of the biomass of these organisms' cultures. As it well known, essential amino acids are important regulators of the main metabolic pathways required for support, growth, reproduction and immunity in all groups of animal organisms [22, 23]. Providing of animals (fodder zooplankton, fish or other farm animals) with feeds characterized by high content of essential amino acids helps to increase protein retention value [24, 25].

The analysis of the amino acid composition in investigated crustaceans cultivated with the use of different yeast species has shown the differences in the ratio of proteinogenic amino acids in *M. macrocopa* (Table 2).

Table 2. Amino acid composition (%) of *M. macrocopa* using different yeast species.

Amino acids	*M. macrocopa* with *S. cerevisiae*	*M. macrocopa* with *R. glutinis*
Lys	7.62 ± 0.680	8.94 ± 0.708
His	2.93 ± 0.292	2.24 ± 0.218
Arg	7.03 ± 0.717	2.04 ± 0.187*
Asp+Asn	9.25 ± 1.009	4.48 ± 0.444*
Thr	6.55 ± 0.619	5.87 ± 0.406
Ser	5.54 ± 0.455	3.14 ± 0.259*
Glu+Gln	16.69 ± 1.513	15.99 ± 1.622
Pro	3.37 ± 0.264	4.30 ± 0.410*
Gly	4.41 ± 0.437	5.84 ± 0.409*
Ala	6.08 ± 0.510	7.99 ± 0.803*
Cys2	1.85 ± 0.165	4.55 ± 0.376*
Val	5.73 ± 0.430	6.61 ± 0.485
Met	1.02 ± 0.076	1.36 ± 0.122*
Ile	4.12 ± 0.365	6.55 ± 0.707*
Leu	8.71 ± 0.701	10.96 ± 1.154*
Tyr	3.59 ± 0.335	2.72 ± 0.210*
Phe	5.52 ± 0.503	6.42 ± 0.542

** Note: All differences are significant at p≤0.05*

Due to the fact, that lysine is one of the most limiting essential amino acids in many types of protein raw materials used in the production of feeds for fish, lysine-rich diet is highly expensive [19, 26]. The lack of lysine causes significant violations in the development of skeletal elements in the early ontogenesis of fish. The role of lysine in nitrogen exchange and osmoregulation of fish is also important [26, 27]. In addition, lysine, along with methionine, participates in the synthesis of carnitine, thus influencing lipid metabolism [26].

It is known that *S. cerevisiae* yeast biomass is characterized by a low content of methionine and histidine [1], which explains the low level of these amino acids in the organisms grown using *S. cerevisiae* (Table 2). It is well known that methionine together with serine in the fish organism participates in the formation of cysteine, thus compensating for the deficit of the last one [28]. Lack of the total content of methionine and cysteine in the diet leads to a decrease in the overall viability of fish [1]. Also methionine and lysine positively influence fish growth and feed ingestion [29]. In addition, it has been shown that sulfur-containing amino acids, including methionine and its derivatives, could affect immune responses by metabolizing of glutathione, taurine and homocysteine through altering the redox state of immune cells and alleviating inflammatory

reactions [30]. The use of *R. glutinis* yeast instead of *S. cerevisiae* in the cultivation of fodder zooplankton can increase the lysine share in the amino acid profile of both *M. macrocopa* (Table 2) and *Simocephalus vetulus* [31].

Feeding of zooplankton with carotene-synthesizing yeast was accompanied by an increase in the level of isoleucine and leucine also. As was previously shown, the derivatives of these amino acids, in particular β-hydroxyl-β-methyl-butyrate as a leucine metabolite, had the stimulating effects on the growth and immune system state of some fish species [32, 33]. For example, branched-chain amino acids, namely valine, leucine and isoleucine, are important in the work of immune cells because of their important role in the synthesis of proteins, including various antibodies, immunoglobulins and acute phase proteins [34]. Accordingly, the use of live feeds enriched with these amino acids provided a high rate of growth processes in fish fry and improved the functioning of the immune system of organism.

The content of certain essential amino acids, in particular histidine, lysine, valine, phenylalanine and threonine, remained virtually unchanged, their concentrations in the live feeds were within normal limits [27]. Thus, in experiments conducted on carp fry, it was shown that the shortage of histidine content not only limited the fish growth rate, but also caused the increasing of osmotic fragility of red blood cells, reduced resistivity to hypoxia and inhibited the activity of antioxidant enzymes [35]. Lysine also corresponded to improvement of haematological parameters [36]. Protecting cells from the detrimental effects of oxidative stress is ensured by the sufficient content of valine in the diet [37]. A slight decrease in tyrosine in the feeds grown on carotene-synthesizing yeast was not critical, since due to the sufficient amount of phenylalanine its content was partially compensated [24]. Phenylalanine is involved in the synthesis of tyrosine. Accordingly, the deficiency of phenylalanine causes the tyrosine deficiency. Tyrosine is a common predecessor for hormones and neurotransmitters, including thyroxin, triiodothyronine, adrenaline, norepinephrine, dopamine and melanin [38]. It is especially important for early stage fish larvae to be provided by sufficient amounts of threonine, a deficiency of which affects the secretory activity of the gastrointestinal tract. This is due to the fact that threonine is a component of mucin in which its share reaches 26% of the total mass fraction of amino acids [25]. A strong deficiency of threonine is especially observed after using of feed produced from plant raw material. The application of both investigated yeast species provides a sufficient level of threonine accumulation in the live fodder organisms (Table 2) [31].

It should be noted that the substitution of traditional yeast *S. cerevisiae* into carotene-producing yeast *R. glutinis* in the process of *M. macrocopa* cultivation leads to a decrease in the share of arginine in the amino acid profile of cladocerans (Table 2). The urea cycle is a pathway for arginine synthesis. In *Cladocera* organisms, as in freshwater fish, the activity of the urea cycle is very low, since these animals are mostly ammoniotelic animals, excreting mainly ammonia with some urea [39]. So that arginine deficiency swiftly affects growth and protein retention in *Moina* and fish. However, on the example of the channel catfish, it has been shown that dietary glutamate may be used for endogenous synthesis of arginine, especially when arginine is deficient in the diet [40]. However, some scientists have questioned the possibility of the existence of such a pathway in fish [38]. Even under the conditions of such transformations, it would cause additional energy expenses, which can not but affect growth rates.

The effectiveness of the live feeds use during fish larvae feeding depended on the balance of their amino acid composition. In particular, the content of non-essential amino acids in feeds should not exceed 50% of their total mass [1]. As can be seen from the obtained results (Table 2), the total share of essential amino acids in the zooplankton using both yeast species is about 50% (tryptophan was not taken into account). Previously conducted investigations on the effect of carotene-producing yeast upon the amino acid composition of other *Cladocera* crustaceans showed a similar tendency to balancing the content of non-essential and essential amino acids in the fodder organisms [31].

4. Conclusion

The use of *R. glutinis* as a fodder substrate in the cultivation of crustaceans *M. macrocopa* not only contributes to the accumulation of carotenoids without reducing the growth dynamics of the culture, but also does not lead to a deterioration of the nutritional value of zooplankton. At the same time, an increasing in the mass fraction of methionine, isoleucine and leucine was noted.

Conflict of Interest

The authors declare that there is no conflict of interest.

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