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The effect of different levels of L-isoleucine in compound feeds on the growth of tilapia (*Oreochromis niloticus*)

ABSTRACT

Four diets were developed, including the control (main diet), which actually contained 34.00% crude protein, while the other three contained protein close to this value, taking into account the measurement error, and isoleucine in groups 2, 3 and 4, 4.9%, 8.51% and 12.12%, respectively. The study of the effect of L-isoleucine additives in compound feeds on the growth of tilapia (*Oreochromis niloticus*) showed that varying the concentration of this amino acid has a significant effect on fish growth rates. Adjusting dosages makes it possible to achieve more efficient use of feed in aquaculture conditions. It was found that when the amino acid isoleucine is introduced into the diet, feed conversion decreases by 0.64 points with an additional concentration of 1.42 g/kg of feed (4.9%), by 0.88 points with additional administration of isoleucine by 1.55 g/kg of feed (8.51%), by 0.76 points with an additional concentration of 1.68 g/kg of feed (12.12%). It was shown that the best group with the lowest feed conversion rate was the 3rd group, in which the amino acid isoleucine was introduced into the diet by 8.51% more than in other groups. The optimal level of isoleucine administration in tilapia feed is 1.55 g/kg of feed (8.51%). It was found that the ash content in the carcass of *O. niloticus* increased by 0.8%, fat — by 1% in muscles compared with the control.

Key words: L-isoleucine, Tilapia, influence, feed, aquaculture, growth

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Влияние разных уровней L-изолейцина в комбикормах на рост тилапии (*Oreochromis niloticus*)

РЕЗЮМЕ

Были разработаны четыре рациона, включающие контроль (основной рацион), который фактически содержал 34,00% сырого протеина, в то время как остальные три содержали белок, близкий к данному значению с учетом погрешности измерения, и изолейцин в 2-й, 3-й и 4-й группах 4,9%, 8,51% и 12,12% соответственно. Изучение влияния добавок L-изолейцина в комбикормах на рост тилапии (*Oreochromis niloticus*) показало, что варьирование концентрации этой аминокислоты оказывает значительное влияние на показатели роста рыбы. Корректировка дозировок позволяет достичь более эффективного использования кормов в условиях аквакультуры. Установлено, что при введении в рацион аминокислоты изолейцина конверсия корма снижается на 0,64 пункта при дополнительной концентрации на 1,42 г/кг корма (4,9%), на 0,88 пункта при дополнительной введении изолейцина на 1,55 г/кг корма (8,51%), на 0,76 пункта при дополнительной концентрации на 1,68 г/кг корма (12,12%). Показано, что лучшей группой с самым низким коэффициентом конверсией корма была 3-я группа, в которой аминокислота изолейцин введена в рацион на 8,51% больше, чем в других группах. Оптимальный уровень введения изолейцина в корм для тилапии — 1,55 г/кг корма (8,51%). Было обнаружено, что содержание золы в тушке *O. niloticus* увеличивается на 0,8%, жира — на 1% в мышцах по сравнению с контролем..

Ключевые слова: L-изолейцин, тилапия, влияние, корма, аквакультура, рост

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Introduction

Aquaculture is a key industry in a number of countries and its development will continue as demand for fish and seafood increases¹ [1].

It is important to many coastal communities along the coast of Gibraltar. It extends from the south coast of the UK to the Faroe Islands, Iceland, the Baltic Sea and to the Russian border in the north. With the growing demand for quality fish, the importance of aquaculture could become even more significant in the coming years [2, 3].

Fish consumption in developing countries is projected to increase by 57%, rising from 62.7 million metric tonnes in 1997 to 98.6 million tonnes by 2020 [4]. At the same time, according to projections by A. Tacon and M. Halwart (2007), fish consumption in developed countries will increase by about 4%, from 28.1 million metric tonnes in 1997 to 29.2 million tonnes by 2020 [5].

Similar to traditional forms of livestock production, fish feeding is a key factor in intensive aquaculture as it affects not only production costs but also the amount of waste generated [6]. It is important to know the nutrient requirements of specific fish species and fulfil them with balanced diets and appropriate feeding practices. Research over the last twenty years has greatly increased our understanding of the nutritional requirements of farmed fish [7, 8].

In aquaculture system, 40–60% of costs are associated with fish feed production. Therefore, it is necessary to continue to reduce feed costs in the future [9, 10]. To ensure high quality fish aquaculture products, it is important to focus on the development of technologies to control the amino acid composition of feeds according to the needs of specific fish species [11].

Recent studies allow to accurately determine the optimum level of crude protein addition, as exceeding this level leads to economic losses and environmental damage [12, 13]. Numerous studies have shown that the optimum protein percentage depends on various factors such as fish species, age and physiological condition.

For successful culturing, it is important that fish receive protein in a balanced and continuous manner throughout the rearing period. Water temperature, presence of contaminants and pathogens also significantly affect the ability of fish to digest protein from the feed mixture [14].

Recently, scientists have paid special attention to the study of the influence of branched side chain amino acids on the balancing of diets for different fish species. Of particular interest is the amino acid isoleucine, which has various systemic physiological effects that contribute to the productivity of fish at different stages of ontogenesis.

It is known that fish need twenty amino acids for protein synthesis. They can synthesise ten of them independently if they are not available in the feed, but the other ten amino acids must come from the feed mixture. Deficiency of one or more of these amino acids results in reduced protein synthesis, which in turn causes weight loss [15, 16]. Therefore, there is a need for balanced feed mixtures with high protein content [17–19].

The percentage of branched-chain amino acids (BCAAs) in fish feeds depends on many factors including fish species, growth stage, environmental conditions, protein level in the feed and target parameters such as growth rate and general health. BCAAs are typically found in natural protein

sources such as fish, grains, legumes and supplements. To determine the optimum ratio of these amino acids, a balance between fish needs, production requirements and the nutritional value of feeds must be considered [20–22].

For example, in the early stages of growth, fish may require a higher percentage of branched-chain amino acids to ensure rapid growth and development of healthy tissues. Whereas adult fish may need less of these amino acids, more geared towards maintaining overall health and productivity. The optimal proportions of branched-chain amino acids in the diet also vary among fish species, as different species have their specific protein and amino acid requirements [23].

Although many factors influence the optimal ratio of branched-chain amino acids (BCAAs) in tilapia feed, some studies indicate that it is usually between 20% and 30% of the total protein in the feed. However, other studies and dietary guidelines suggest that the optimal ratio of isoleucine in tilapia feed is often between 4 and 5% of the total protein in the feed. This ratio is considered common and may be suitable for most production conditions [24].

*The aim of the study was to investigate different levels of added branched amino acids, including isoleucine, in the diets of tilapia *O. niloticus* on productive parameters in ontogeny.* Today, industrial cultivation of tilapia implies exclusively cultivated Indian tilapia which show the most productive qualities.

Materials and methods

The experimental work was carried out in 2024 on the basis of the Department of fish breeding of Volgograd State Agrarian University.

Before the beginning of the experiment the fish were acclimatised for a fortnight. 200 *O. niloticus* juveniles with an average body weight of 12.0 ± 0.63 g and body length of 9.0 ± 0.37 cm participated in the experiment.

The fish were divided into 4 groups with the same number of individuals. Fish weight was monitored from the time of stocking in the tanks. Fish were reared in rectangular glass aquaria of 100 litres filled with dechlorinated water¹. The daily feeding rate was determined according to fish body weight and water temperature in accordance with the rearing technology [25, 26]. Feeding of fish was carried out manually 4 times a day.

According to the experiment variants, the groups were reared with the same planting density.

The experimental conditions included maintaining the same level of cleanliness of the internal and external environment in all experimental groups. Individual lights were installed above each aquarium where Indian tilapia were grown. Water aeration was used throughout the experiment using Hidom AP-1200 pumps, 13 W and 800 l/h (China).

Feed residues accumulating on the filters were removed manually several times a day. The hydrochemical condition of the water was monitored daily. The water in the aquarium was changed daily at 10–20% manually. Water temperature was maintained at 28 ± 1 °C using thermostatically controlled immersion heaters such as the 300 W adjustable glass heater SHANDA SDH-318, manufactured by SHANDA (China). Oxygen content, water temperature and pH were recorded daily using a professional digital dissolved oxygen meter (oximeter) AR8406 (Chanfong, China).

¹ Mobsby D., Steven H. A., Curtotti R. Australian fisheries and aquaculture outlook 2020. <https://doi.org/10.25814/5e4377eb3eeea2>

² ГОСТ 34088-2017 Руководство по содержанию и уходу за лабораторными животными. Правила содержания и ухода за сельскохозяйственными животными (GOST 34088-2017 Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of farm animals).

Water quality was monitored daily using VladOx screening tests from MEDOSA LLC in Russia to assess pH, ammonia, nitrite, nitrate and carbonate hardness levels. Water quality parameters were within the desired range for tilapia: pH 6.9–8.0, ammonia (NH₃) 0.08–0.21 mg/l, nitrite (NO₂) 0.17–0.36 mg/l, nitrate (NO₃) 4.28–5.71 mg/l, and dissolved oxygen within 5.9–7.4 mg/l [27, 28].

The main tests were carried out at the collective use Centre: amino acid analysis according to GOST 32195³, chemical parameters of fodder were determined according to standardized methods: moisture according to GOST R 57059⁴, crude ash was determined according to GOST 26226⁵, crude protein GOST 13496.4⁶, crude fat according to GOST 7636⁷.

The scheme of the main parameters of the experiment is presented in Table 1.

Results and discussion

Four diets were formulated including a control diet (main diet) containing actually 34.00% crude protein, while the other three diets contained protein close to this value considering the measurement error.

Growth performance for *O. niloticus* fed according to the four diets is presented in Table 4.

No mortalities were recorded during the study period. A statistically significant difference ($p < 0.05$) was found in terms of final mean body weight and body length in fish that received diets 1 (control diet), 2, 3 and 4 compared to the control group.

The highest mean body weight was recorded in fish that were additionally fed isoleucine at 8.51% level, being 42.59 g.

Figure 1. Mean body length of fish

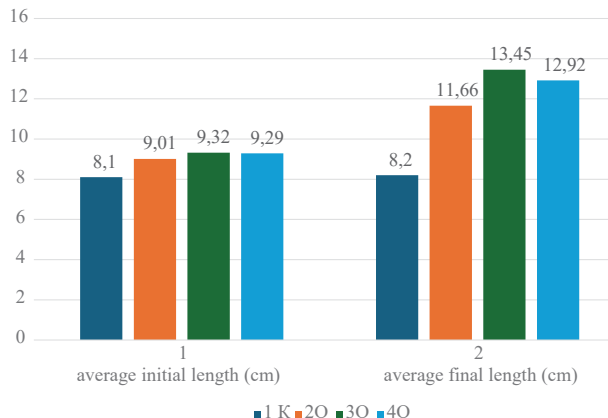


Table 4. Dynamics of body weight, feed conversion when using experimental diets of *O. niloticus* (M — mean value ± SD standard deviation, n = 5)

Indicators	Diet options			
	1 (control)	2	3	4
Average initial weight (g)	12.94 ± 2.16	12.65 ± 1.12	12.56 ± 1.18	12.66 ± 0.93
Average final weight (g)	30.46 ± 3.98	35.64 ± 1.7 ^a	42.59 ^a ± 1.58 ^{a, b}	41.97 ^a ± 1.65 ^{a, b, c}
Increase in body weight (g)	17.52 ± 0.55	22.99 ± 0.06 ^a	29.53 ^a ± 0.61 ^{a, b}	27.31 ^b ± 1.57 ^{a, b, c}

Note: Validity ($p > 0.05$) here and below: a — to 1st control group; b — 3rd to 2nd group; c — 4th to 2nd group; d — 4th to 3rd group.

Table 1. Scheme of the main parameters of the experiment

Indicators	Water volume, l	Period studies, days	Initial juvenile weight, g	Density planting density of fish, pcs/m ²	Feeding method
Option 1: con	100	14/30	12/18	300/250	Manually
Option 2: Ex	100	14/30	12/21	300/250	Manually
Option 3: Ex	100	14/30	12/27	300/250	Manually
Option 4: Ex	100	14/30	12/25	300/250	Manually

Table 2. Summaries the analysis of the main food ingredients carried out before the experimental diets were formulated. Composition of the basic ration by mass fraction, %

Nutritional parameters	Theoretically — Recipe	Fact — after analysis
Damp	13.80	12.60
Crude protein	33.10	34.00
Crude fat	8.00	9.65
Crude fibre	4.84	4.81

Table 3. Content and ratio of main amino acids with branched side chain of protein component of the basic diet and variants

Indicators	Isoleucine mass fraction, %	Mass fraction of valine, %	Mass fraction of leucine, %	Ratio Iso:Val:Leu
Option 1: con	1.29 = 1	1.51 = 1	2.31 = 1	1:1:2
Option 2: Ex	4.9 = 3.79	1.51 = 1	2.31 = 1	3:1:2
Option 3: Ex	8.51 = 6.59	1.51 = 1	2.31 = 1	6:1:2
Option 4: Ex	12.12 = 9.39	1.51 = 1	2.31 = 1	8:1:2

The fish supplemented with isoleucine at 12.12% and 4.9% levels had a mean body weight of 41.97 g and 35.64 g, respectively, while the control group with 1.29% isoleucine had a mean body weight of 30.46 g (Table 4).

As shown in the above figure, the final body length of the fishes ranged from 10.32 to 12.92 cm.

The highest length was recorded in group 3 and was 13.45 cm, while the lowest value was recorded in control groups 2 and 4 where the length was 10.32 cm and 11.66 cm respectively and in group 4 where the length was 12.92 cm.

The data presented in Table 4 explains that the highest weight gain among the experimental groups was recorded in group 3 which was 29.53 g which was slightly higher than the gain compared to the other two groups of group 2 and 4 where the gain was 22.99 g and 27.31 g respectively compared to the control group where the weight gain was 17.52 g.

The results for specific growth rate (SGR%) further confirmed this trend, reflecting the highest specific growth rate of SGR in group 3, which was 1.45%. In groups 4 and 2, the specific growth rate was slightly lower at 1.42% and 1.23%, respectively, while the control group had 1.05%.

The SGR values for groups 2 and 1 were 1.23% and 1.19%, respectively, and were statistically significantly different ($p < 0.05$) from all other groups.

The increase in body weight was also significantly higher in groups 3 and 4 compared to the other groups. Fish fed experimental feeds (Groups 3 and 4) were well consumed except for fish fed diets from Groups 1 and 2 which contained 34.0% protein and isoleucine at 8.51%, respectively. With these diets, the total feed intake per fish was 47.76 g

³ ГОСТ 32195-2013 Корма, комбикорма. Метод определения содержания аминокислот (ГОСТ 32195-2013 Feed, compound feed. Method for determination of amino acid content).

⁴ ГОСТ Р 57059-2016 Корма, комбикорма, комбикормовое сырье. Экспресс-метод определения влаги (ГОСТ Р 57059-2016 Feed, compound feed, feed raw materials. Express method for determining moisture).

⁵ ГОСТ 26226-95 Корма, комбикорма, комбикормовое сырье. Методы определения сырой золы (ГОСТ 26226-95 Feed, compound feed, feed raw materials. Methods for the determination of crude ash).

⁶ ГОСТ 13496.4-2019 Корма, комбикорма, комбикормовое сырье. Методы определения содержания азота и сырого протеина (ГОСТ 13496.4-2019 Feed, compound feed, feed raw materials. Methods for determining nitrogen and crude protein content).

⁷ ГОСТ 7636-85 Рыба, морские млекопитающие, морские беспозвоночные и продукты их переработки. Методы анализа (ГОСТ 7636-85 Fish, marine mammals, marine invertebrates and products of their processing. Methods of analysis).

and 45.50 g, respectively, which was significantly lower ($p < 0.05$) compared to that of groups 3 and 4, which were 48.341 g and 48.21 g, respectively (Table 5).

The best values of feed conversion ratio (FCR) to body weight were 1.65, 1.77 and 1.89 for groups 3, 4 and 2, respectively, compared to the control group where FCR was 2.53. Protein efficiency ratio (PER) also varied significantly among the diets used, following a similar trend. Fish receiving dietary option 3 had the best PER (1.81), followed by options 4 and 2 (1.80 and 1.73, respectively), with insignificant differences between groups.

Thus, the addition of the amino acid isoleucine in the diet resulted in a 0.64 point decrease in feed conversion at an additional concentration of 4.9% in feed, 0.88 points at an additional 8.51% isoleucine in feed, and 0.76 points at an additional 12.12% in feed. The best group with the lowest conversion was group 3 with the addition of isoleucine at 8.51% in feed.

Further increase in isoleucine content in the diet for fish of the 4th experimental group (12.12%) showed that the conversion rate remained in the same range as that of the 3rd experimental group or slightly exceeded it. Protein productivity indices also increased slightly when fish were fed this diet. They were 25.58% and 24.88% in group 3 and 4, respectively, and the difference between treatments was significantly significant ($p < 0.05$) (Table 5).

The chemical composition of fish carcasses fed under the experimental diets is presented in Table 6.

The chemical composition of *O. niloticus* carcass also differed in different components for different experimental diets. Fish given diets with isoleucine concentration of 8.51% and 12.12% in groups 3 and 4, respectively, had significantly higher carcass protein content (16.40% and 15.49%, respectively). Body fat content varied significantly. Higher fat content was observed in fish of group 3 at 4.90% ($p < 0.05$), while the diets of control and 2nd experimental groups had lower fat content. As shown in Table 6, the crude ash content of the body differed significantly among the diet variants. Significantly higher ash content was observed in fish of groups 3 and 4, 4.27% and 4.03%, respectively ($p < 0.05$), while ash level was lower in diets of control and 2nd experimental groups by 3.92% and 3.46%, respectively.

Table 5. Performance indices using experimental diets of *O. niloticus* (M – mean value ± SD standard deviation at $n = 5$)

Indicators	Diet options			
	1 (control)	2	3	4
SGR %1	1.05 ± 0.13	1.23 ± 0.13 ^a	1.45 ± 0.15 ^{a, b}	1.42 ± 0.14 ^a
Weight gain %	135.39 ± 2.85	183.76 ± 4.31	239.09 ± 4.29 ^{a, b}	231.52 ± 4.02 ^{a, b}
Total feed consumption, g/fish	45.50 ± 1.71	47.76 ± 1.73	48.34 ± 1.39 ^{a, b}	48.21 ± 1.68
FCR ²	2.53 ± 0.18	1.89 ± 0.27 ^a	1.65 ± 0.27 ^{a, b}	1.77 ± 0.17 ^{a, b}
PER ³	1.65 ± 0.35	1.73 ± 0.30	1.81 ± 0.35 ^{a, b}	1.80 ± 0.27 ^{a, b}
PPV (%) ⁴	17.54 ± 1.53	20.81 ± 1.20 ^a	25.58 ± 1.00 ^{a, b}	24.88 ± 1.62 ^a

Note: Hereinafter: 1SGR: [Final body weight (g) – initial body weight (g)] / period of experiment (days) × 100; 2FCR: feed intake (g) / body weight gain (g); 3PER: body weight gain (g) / protein intake (g); 4PPV (%): protein content (g) / total protein intake (g) = 100.

Table 6. Composition of *O. niloticus* carcasses in diets with added amino acids (M – mean value ± SD standard deviation at $n = 5$), %

Indicators	Diet options			
	1 (control)	2	3	4
Damp	74.25 ± 0.45	74.57 ± 0.71	74.51 ± 0.63	75.23 ± 0.63
Dry matter	25.75 ± 0.46	25.43 ± 0.31	25.49 ± 0.33	24.77 ± 0.13
Organic matter	22.29 ± 0.33	21.51 ± 0.21	21.22 ± 0.20	20.74 ± 0.05
Crude protein	14.40 ± 0.20	15.12 ± 0.48	16.40 ± 0.55	15.49 ± 0.30
Crude fat	2.69 ± 0.52	2.85 ± 0.62 ^a	4.90 ± 0.65 ^{a, b}	3.61 ± 0.65 ^{a, b}
Crude ash	3.46 ± 0.13	3.92 ± 0.10 ^a	4.27 ± 0.13 ^{a, b}	4.03 ± 0.08 ^{a, b}

Conclusion

It was found that addition of amino acid isoleucine to the diet reduced feed conversion by 0.64 points when 1.42 g/kg feed was added (representing 4.9%), 0.88 points when 1.55 g/kg feed was added (representing 8.51%), and 0.76 points when 1.68 g/kg feed was added (representing 12.12%).

It was also revealed that group 3 in which isoleucine was added to the diet at 1.55 g/kg feed (8.51%) had the best feed conversion ratio compared to other groups.

The optimum level of isoleucine supplementation in tilapia feed is 1.55 g/kg feed (8.51%).

Isoleucine supplementation in tilapia feed was found to increase ash content by 0.8% and fat content by 1% in *O. niloticus* carcass muscle compared to control. It also increases the energy value of the meat and does not affect its biological value.

All authors bear responsibility for the work and presented data. All authors made an equal contribution to the work. The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism. The authors declare no conflict of interest.

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