STUDY ON THE USE OF NEW FEED SOURCES IN FISH NUTRITION

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Abstract
The study aimed to evaluate the potential of alternative feed ingredients in common carp (Cyprinus carpio) nutrition within a recirculating aquaculture system. Two experimental approaches were carried: partial replacement of fishmeal with Black Soldier Fly (Hermetia illucens) larvae meal and supplementation with thyme essential oil. Ninety carp specimens were distributed in experimental groups and fed diets formulated with varying levels of insect meal (10% and 30%) or with thyme oil (0.2%). Growth performance, feed conversion, and muscle biochemical composition were analyzed. Results showed that the inclusion of insect meal improved lipid content and maintained growth performance comparable to the control diet, while thyme oil supplementation had positive effects on feed intake and health status. These findings highlight the potential of novel, sustainable feed ingredients to partially replace traditional fishmeal and support ecological and cost-effective aquaculture practices.

Key words: *aquaculture, carp, insect meal, Black Soldier Fly, thyme oil*

INTRODUCTION

Aquaculture is one of the fastest-growing sectors in global food production, playing a vital role in ensuring food security and economic development worldwide [1]. In the European Union, aquaculture is highly concentrated in certain regions. In 2023, production reached nearly 1.1 million tons of live weight, generating an estimated value of €4.8 billion, with Spain, France, Greece, and Italy accounting for around two-thirds of the total output [2].

In Romania, aquaculture represents a significant share of total fisheries production, contributing approximately 77% of the national output. The sector is almost entirely based on freshwater pond farming, with common carp (*Cyprinus carpio*) being the dominant species due to its adaptability, economic value, and strong market demand [3].

Despite its growth, aquaculture still depends heavily on fishmeal as the main protein source in feed formulations. However, the reliance on fishmeal has become increasingly unsustainable, given its high cost, environmental impact, and the overexploitation of marine resources [4]. As a result, alternative protein sources are needed to support sustainable aquaculture development.

Among these alternatives, insect meals, especially Black Soldier Fly (*Hermetia illucens*) larvae meal, have received growing attention. Research from Africa, Asia and Europe indicates that BSF meal provides a high-quality protein source, and its inclusion in aquafeeds can enhance growth performance, feed efficiency, antioxidant capacity, and immune responses in fish [5, 6, 7].

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In addition to alternative proteins, functional feed additives derived from plants are increasingly used to improve fish health and product quality. Essential oils such as thyme oil are recognized for their antimicrobial, antioxidant, and immunomodulatory properties. Several studies have reported positive effects of thyme oil supplementation on growth performance, feed utilization, and immune responses in different aquaculture species [9, 10].

In this context, the present study aimed to investigate the combined effects of partial fishmeal replacement with BSF larvae meal and dietary supplementation with thyme essential oil on the growth performance and biochemical composition of common carp (*Cyprinus carpio*).

MATERIAL AND METHOD

Experimental conditions and fish distribution

In the spring of 2023, at the Research and Development Station for Aquaculture and Aquatic Ecology Iași, a total of 90 common carp (*Cyprinus carpio*) juveniles were selected from a group of 500 individuals, with an initial body weight of 100-106 g. The experimental batch was transferred to the station's recirculating aquaculture system (RAS) and acclimated for 10 days prior to the start of the trial.

The RAS was equipped with water tanks, drainage systems, feeding devices, a mechanical filter, a biological filter, a UV filter, recirculation pumps, automation systems, and oxygen, temperature, and pH sensors (Figure 2). Throughout the experiment, water quality was carefully monitored with the Hanna spectrophotometer and the in-tank sensors to ensure optimal rearing conditions. Water parameters (ammonia, nitrite, nitrate) were measured using a Hanna Iris spectrophotometer with Hanna reagent kits (Figure 1): Hach HQ30d for dissolved oxygen and Hach HQ11d for pH and conductivity. Water parameters are presented in table 1.

Fig. 1 Hanna Iris Spectrophotometer

Diet preparation and ingredients used

To prepare the diets for common carp, the ingredients, such as cereals and protein meals, were ground in a mill to obtain a fine powder. The mixture was then processed through an extruder, where it was subjected to cooking at high temperature and pressure to improve digestibility. Afterwards, the pellets were shaped and dried to ensure proper storage (Figure 4). In the experimental diets, Black Soldier Fly (*Hermetia illucens*) larvae meal was included as an excellent and sustainable source of protein and lipids. In addition, thyme essential oil was used, given its antioxidant and antimicrobial properties, which contribute to maintaining fish health.

In the experimental studies on diet formulation for common carp, a DA 7250 NIR analyzer (Figure 3) was used to rapidly and accurately evaluate the nutritional composition of the feeds, including ingredients such as Black Soldier Fly larvae meal and thyme essential oil. This instrument enabled the measurement of essential parameters, such as protein, fat, fiber, and other nutrients, in a very short time, thereby contributing to the optimization of diet formulation.

In the first experiment (Table 2), three feed diets were prepared: one control diet (without Black Soldier Fly) and two experimental diets containing different inclusion levels of BSF meal: V1(10%) and V2(30%). The control diet consisted of 400 g/kg fishmeal, 300 g/kg meat meal, 270 g/kg sunflower meal, and 30 g/kg soybean oil. All ingredients used, such as protein meals, maize, wheat, peas, soybean oil, and dried distillers' grains with solubles (DDGS), are commonly included in carp diets.

In the second experiment (Table 3) only two feed diets were prepared: one control diet and E-TO diet with 0.2 % thyme (*Thymus vulgaris*) essential oil. Mostly the same locally sourced ingredients were used, with the exception of insect meal.

Fig. 2 - Recirculating aquaculture system (RAS) tanks with the experimental batch of fish

Table 1 Water quality parameters in carp rearing tanks during the experiment

Parameter	Range	Reference values [11]
Physical parameters	-	-
Temperature (°C)	21.5 - 25.8	22-26
pH	7.7 – 8.2	6.5 - 8.5
Dissolved oxygen (mg L^{-1})	7.69 - 8.51	> 5.0
Chemical parameters	-	-
Total hardness (mg $CaCo_3$ L^{-1})	111 - 128	50 - 400
Calcium (mg Ca^{2+} L^{-1})	79 – 104	4 - 160
Magnesium (mg Mg^{2+} L^{-1})	71 – 89	< 15
Iron (mg Fe L^{-1})	0.01 – 0.031	< 0.15
Nitrogen compounds	-	-
Total ammonia (mg NH_3 L^{-1})	0.00 - 0.04	< 0.01
Ammonium (mg NH_4^+ L^{-1})	0.00 - 0.05	≤ 0.50
Nitrite (mg NO_2^- L^{-1})	0.06 - 0.11	< 0.10
Nitrate (mg NO_3^- L^{-1})	3.3 – 10.2	0 - 400

Table 2 Ingredients and proximate composition of the experimental diets with BSF meal for *Cyprinus carpio*

Parameter	Control (0% BSF)	V1 (10% BSF)	V2 (30% BSF)
Ingredient composition (g/kg^{-1})	-	-	-
Fish meal	400	400	200
Meat meal	300	240	100
Insect meal	0	100	300
Peas	0	30	50
DDGS[1]	0	20	30
Sunflower meal	270	180	200
Soybean oil	30	20	20
Wheat	0	10	20
Corn	0	0	30
Chemical composition (g/kg^{-1} as fed basis)	-	-	-
Moisture	103.3±0.8	100.6±0.8	101.5±1.1
Crude protein	318.4±1.8	311.3±2.2	308.1±2.3
Crude fat	74.4±0.2	80.9±0.4	88.3±0.4
Crude fiber	75.7±0.6	69.4±0.4	76.2±0.3
Starch	77.4±1	139.8±1.9	122.3±2.6
Ash	132.2±1	136.8±1	139.8±1.3
Total sugars	63.7±1.7	64.1±0.9	61.3±1
Calcium	42±1.3	30.1±0.7	33±0.6
Phosphorus	15.4±0.3	15.1±0.3	17.2±0.1

Note: [1] Dried Distillers Grains with Solubles

Table 3 Ingredients and proximate composition of the experimental diets with thyme oil 0.2 % for *Cyprinus carpio*

Parameter	Control	E-TO (thyme oil 0,2 %)
Ingredient composition (g/kg^{-1})	-	-
Fish meal	400	400
Meat meal	290	290
Sunflower meal	230	230
Peas	20	20
DDGS[1]	10	10
Soybean oil	30	28
Wheat	10	10
Corn	10	10
Thyme oil	0	2
Chemical composition (% as fed basis)	-	-
Moisture	10.56±0.05	9.86±0.11
Crude protein	30.65±0.26	30.08±0.18
Crude fat	8.23±0.10	8.36±0.07
Crude fiber	7.26±0.05	7.26±0.12
Starch	5.3±0.24	5.3±0.23
Ash	14.33±0.05	13.46±0.11
Total sugars	6.35±0.18	5.94±0.20
Calcium	4.36±0.06	4.44±0.06
Phosphorus	1.656±0.03	1.86±0.04

Note: [1] Dried Distillers Grains with Solubles

Fig. 3 - DA 7250 rapid NIR analyzer (PerkinElmer)

Fig. 4 Equipment used for the preparation of experimental diets
A – Mill, B – Extruder, C – Pelletizer

Evaluation of Growth Performance

To determine the growth parameters, the fish were measured (in cm) and weighed (in g) three times during the indicated growth period. Based on these measurements, the main growth indices were determined and calculated, and were as follows:

- IBW (initial body weight, g);
- FBW (final body weight, g);
- WG (weight gain, g) = FBW − IBW;
- SGR (specific growth rate, % day^{-1}) = (ln FBW − ln IBW)/days of experiment × 100;
- FCR (feed conversion ratio, g/g) = Feed intake (g)/WG;
- CF (condition factor) = FBW/body length^3 × 100;
- PER (protein efficiency ratio) = WG/Protein intake (g);
- LER (lipid efficiency ratio) = WG/Lipid intake (g);
- HSI (hepatosomatic index, %) = 100 × [final liver weight (g)/final body weight (g)];

VSI (viscerosomatic index, %) = 100 × [final visceral weight (g)/final body weight (g)].

RESULTS

Evaluation of Growth Performance

The growth performance of carp is summarized in table 4 and table 5 for both experiments. The results demonstrate a clear trend in the growth performance of common carp in response to varying levels of Black Soldier Fly larvae flour inclusion in their diets. In table 4 the initial body weight (IBW) indicated that the mean initial weight of carp was similar across all three experimental groups: control (106.84 ± 2.79 g), V1 (10% BSF) – 114.10 ± 2.53 g, and V2 (30% BSF) – 114.56 ± 2.04 g.

Final body weight (FBW) showed significant differences among the experimental variants. Carp fed the V2 diet (30% BSF) reached the highest final weight (348.15 ± 10.79 g), significantly higher than the control group (298.63 ± 10.20 g). The V1 diet (10% BSF) resulted in an intermediate value (322.67 ± 9.75 g), which did not differ significantly from either the control or V2. These results suggest that a higher inclusion level of Black Soldier Fly larvae meal may have a beneficial effect on carp growth. For weight gain (WG), a clear difference was observed between the control diet and the V2 diet with 30% BSF Condition factor (CF) did not show significant differences, indicating good overall fish health. Feed conversion ratio (FCR) did not differ significantly among groups, although the V2 diet showed a lower value compared to V1 and the control, suggesting better feed utilization in carp fed V2. Other growth indicators did not present statistically significant differences.

Regarding the growth parameters presented in table 5, no significant differences were recorded, since volatile oils are not used as growth promoters but rather as immunomodulators to enhance fish immunity.

Proximate Composition of Fish Meat

The biochemical composition of common carp muscle (Figure 5) with diets containing BSF larvae meal and thyme oil is summarized in table 6 and 7.

Fig. 5 Muscle tissue samples from carp fed BSF diets

According to the data in table 6 moisture values showed only minor differences between groups, with the highest level being recorded in V2 (30% BSF) at 76.5 ± 0.32[a]. No significant statistical differences were observed in protein content, which remained constant across groups.

In terms of lipid levels, higher values were recorded in the control and V1 groups compared to V2, where a significantly lower value was found. Ash content showed higher statistical values in the control and V1 groups, but not in V2, where the level was relatively low. Regarding collagen concentration, the results suggest that a 10% inclusion level of BSF meal has a positive effect on collagen synthesis, representing an optimal proportion, whereas the 30% BSF inclusion appears to negatively influence collagen formation.

Black Soldier Fly larvae meal contains a considerable amount of chitin, a structural polysaccharide in the insect exoskeleton. At moderate inclusion levels (e.g., 10%), chitin may act as a dietary fiber with beneficial effects on gut function and may indirectly stimulate collagen synthesis. However, at higher inclusion levels (e.g., 30%), the excess chitin can reduce protein digestibility and amino acid availability, leading to a negative effect on collagen deposition in fish muscle.

Table 4 Growth performance parameters of *Cyprinus carpio* fed BSF experimental diets

Parameters	Control	V1 (10% BSF)	V2 (30% BSF)	p-value
IBW (g)	106.84±2.79^{ns}	114.1±2.53^{ns}	114.56±2.04^{ns}	0.044
FBW (g)	298.63±10.2^{b}	322.67±9.75^{ab}	348.15±10.79^{a}	0.01
WG (g)	192.79±2.1^{b}	208.57±4.23^{ab}	233.59±13.86^{a}	0.001
CF	1.87±0.03^{ns}	1.83±0.04^{ns}	1.65±0.04^{ns}	0.051
FCR (g/g)	2.83±0.06^{ns}	2.83±0.05^{ns}	2.56±0.06^{ns}	0.071
SGR (% day^{-1})	10.13±2.51^{ns}	10.33±2.57^{ns}	11.10±2.99^{ns}	0.9
RG (g/g day^{-1})	0.17±0.04^{ns}	0.17±0.05^{ns}	0.20±0.06^{ns}	0.736
PER (g/g)	0.58±0.01^{ns}	0.59±0.01^{ns}	0.64±0.02^{ns}	0.056
LER (g/g)	2.43±0.05^{ns}	2.45±0.05^{ns}	2.38±0.06^{ns}	0.52
VSI (%)	9.97±0.75^{ns}	11.58±1.13^{ns}	11.12±1.09^{ns}	0.74
HSI (%)	0.28±0.03^{ns}	0.22±0.03^{ns}	0.29±0.02^{ns}	0.37

Note: IBW-initial body weight, FBW-final body weight, WG-weight gain, FCR-feed conversion ratio, CF-condition factor, PER-protein efficiency ratio, LER-lipid efficiency ratio, HIS-hepatosomatic index, and VSI-viscerosomatic index. Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences

Table 5 Growth performance parameters of *Cyprinus carpio* fed the experimental diet with 0.2% thyme essential oil

Parameters	Control	E-TO (thyme oil 0.2 %)	p-value
IBW (g)	100.35 ± 3.59^{ns}	104.32 ± 2.94^{ns}	0.285
FBW (g)	258.18 ± 13.1^{ns}	288.31 ± 11.54^{ns}	0.181
WG (g)	157.73 ± 19.3^{ns}	185.19 ± 13.0^{ns}	0.578
CF	1.84 ± 0.04^{ns}	1.78 ± 0.03^{ns}	0.64
FCR (g/g)	2.74 ± 0.07^{ns}	2.65 ± 0.03^{ns}	0.599
SGR (% day^{-1})	9.53 ± 2.25^{ns}	10.07 ± 2.49^{ns}	0.996
RG (g/g day^{-1})	0.14 ± 0.04^{ns}	0.16 ± 0.04^{ns}	0.986
PER (g/g)	0.88 ± 0.14^{ns}	0.89 ± 0.01^{ns}	0.878
LER (g/g)	2.78 ± 0.14^{ns}	3.24 ± 0.04^{ns}	0.35
VSI (%)	11.80 ± 0.75^{ns}	11.25 ± 1.4^{ns}	0.365
HIS (%)	0.23 ± 0.05^{ns}	0.27 ± 0.03^{ns}	0.198

Note: IBW-initial body weight, FBW-final body weight, WG-weight gain, FCR-feed conversion ratio, CF-condition factor, PER-protein efficiency ratio, LER-lipid efficiency ratio, HIS-hepatosomatic index, and VSI-viscerosomatic index. Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences.

Table 6 Biochemical composition of common carp muscle fed with experimental diets containing BSF meal

Parameters	Control	V1 (10% BSF)	V2 (30% BSF)	p-value
Moisture (%)	74.56 ± 0.22^{b}	75.5 ± 0.44^{b}	76.5 ± 0.32^{a}	0.02
Protein (%)	18.3 ± 0.19^{ns}	18.3 ± 0.09^{ns}	18.74 ± 0.18^{ns}	0.15
Fat (%)	2.06 ± 0.11^{a}	2.2 ± 0.11^{a}	1.54 ± 0.13^{b}	0
Ash (%)	1.71 ± 0.11^{a}	2.35 ± 0.19^{a}	0.89 ± 0.06^{b}	0
Collagen (%)	0.81 ± 0.09^{b}	1.28 ± 0.09^{a}	0.24 ± 0.07^{c}	0
Salt (%)	0.44 ± 0.19^{ns}	0.9 ± 0.08^{ns}	0.6 ± 0.18^{ns}	0.39

Note: Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences

Table 7 Biochemical composition of common carp muscle fed an experimental diet supplemented with 0.2% thyme oil

Parameters	Control	E-TO (thyme oil 0,2 %)	p- value
Moisture (%)	76.51 ± 0.27^{ns}	75.87 ± 0.27^{ns}	0.572
Protein (%)	15.83 ± 0.09^{b}	16.82 ± 0.23^{a}	0.002
Fat (%)	2.76 ± 0.13^{a}	2.31 ± 0.14^{b}	0.002
Ash (%)	2.38 ± 0.10^{ns}	2.43 ± 0.10^{ns}	0.314
Collagen (%)	1.62 ± 0.10^{b}	1.72 ± 0.06^{a}	<0.001
Salt (%)	0.61 ± 0.09^{ns}	0.49 ± 0.05^{ns}	0.425

Note: Different lowercase letters represent statistically significant differences according to Tukey's test at $p < 0.05$. 'ns' denotes non-significant differences

According to table 7, no statistically significant difference was observed in moisture content. For protein, the group fed the experimental diet supplemented with 0.2% thyme essential oil showed a higher percentage compared to the control group. Fat content was significantly lower in the thyme oil group (2.31 ± 0.14%) compared to the control group (2.76 ± 0.13%), with a statistically significant difference ($p = 0.002$). This result may be attributed to the influence of active compounds in thyme essential oil on lipid metabolism, by reducing fat synthesis from other nutrients such as carbohydrates and by stimulating the utilization of fatty acids as an energy source. Collagen content was significantly higher in the thyme oil group (1.72 ± 0.06%) compared with the control (1.62 ± 0.10%), with a highly significant difference ($p < 0.001$). This effect may be associated with the stimulatory role of the bioactive compounds in thyme essential oil on collagen synthesis.

CONCLUSIONS

The present study aimed to provide new insights into the use of sustainable feed ingredients in common carp (*Cyprinus carpio*) nutrition. The partial replacement of fishmeal with Black Soldier Fly (BSF) larvae meal demonstrated that insect protein can serve as a viable alternative, supporting growth performance while reducing the reliance on conventional marine resources. The results also revealed that moderate inclusion levels (10%) may be more beneficial for certain biochemical traits, such as collagen synthesis, while higher inclusion levels (30%) enhanced final body weight but showed mixed effects on muscle composition.

The supplementation of diets with thyme essential oil (0.2%) did not significantly influence growth parameters, which was expected given the functional role of volatile oils. However, its positive effects on the biochemical profile of fish muscle, including reduced lipid levels and increased collagen content, highlight its potential as a natural immunomodulator and functional additive. Such bioactive compounds may contribute to improving fish health, product quality, and consumer acceptance.

Overall, the findings emphasize the dual value of BSF meal and thyme essential oil: (1) as practical solutions for sustainable aquaculture production and (2) as contributors to reducing environmental impact and dependence on traditional feed resources. From a scientific perspective, this research strengthens the evidence base for incorporating alternative protein and functional additives in carp nutrition, while from a practical perspective, it provides guidance for farmers seeking cost-effective and eco-friendly feeding strategies. Future studies should focus on long-term trials, different inclusion levels, and the synergistic effects of combining insect meal with plant-based bioactive compounds to further optimize fish health and production efficiency.

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