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THE EFFECT OF YARROW (*ACHILLEA MILLEFOLIUM*) SUPPLEMENTED DIET ON GROWTH PERFORMANCE, BIOCHEMICAL BLOOD PARAMETERS AND MEAT QUALITY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* W.) AND GROWTH OF LETTUCE (*LACTUCA SATIVA*) CULTIVATED IN AQUAPONIC RECIRCULATION SYSTEM

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Abstract

Yarrow (*Achillea millefolium*) is medicinal plant used in Bulgarian traditional medicine to improve the condition of the digestive tract and could positively affect digestibility and assimilation of feed nutrients, as well as physiological condition in human and animal organisms. The aim of current study was to find the effect of feed, supplemented with yarrow (*Achillea millefolium*) on growth performance, blood parameters and meet quality in rainbow trout (*Oncorhynchus mykiss* W.) as well as on growth of heads and roots in lettuce (*Lactuca sativa*), raised in the aquaponics recirculation system.

The fish were fed with two feeds: control feed (CF) - without the addition of supplement and experimental feed (EF), with supplementation of the extract of yarrow in quantity of 15 g/kg of feed fed. The initial average weight of fish in CF variant was 114.4 ± 2.72 g and in EF variant was 109.8 ± 2.10 g without differences being statistically significant ($P \geq 0.05$). The continuation of the experiment was 60 days. The average final weight, meat quality, and blood biochemical parameters were measured at the end of the experiment. The blood samples were examined by the colorimetric method with blood analyzer (Mindray SC – 120). The meat quality (moisture %, dry matter %), crude protein content, fat and ash content were determined respectively to Bulgarian State Standards: (BSS)11374-86, BSS-ISO 5983, BSS-ISO 6492, BSS 11374-86). The data were analyzed statistically with Anova single factor Statistica 6.0 software.

The average final weight in trout from the experimental group was higher with 11.0%, compared with the value of this parameter, found for the rainbow trout from the control variant ($P < 0.001$). The aspartate aminotransferase, alkaline phosphatase and cholesterol levels in the blood of fish fed with feed supplemented with yarrow were higher, respectively with 27.4%, 57.2%, and 24.9%, compared with average values in these blood parameters found out for fish from control variant ($P < 0.05$). The parameters of meat quality in fish from experimental groups were not affected significantly from yarrow supplementation in fish feed and were similar to those found in fish from the control group ($P \geq 0.05$). The weight of head and roots in lettuce cultivated in the aquaponics system were respectively 95.4 ± 3.06 g and 26.9 ± 0.82 g at the end of the trial.

The supplemented diet with yarrow affects the growth and biochemical blood parameters (aspartate aminotransferase, alkaline phosphatase and cholesterol) in rainbow trout and did not affect the weight of head and roots in lettuce cultivated in the aquaponics system.

***Key words**: Aquaponics recirculation system, Yarrow supplementation, Rainbow trout, Blood biochemical parameters, Growth, Meat quality.*

1. Introduction

The aquaponics is new technology where cultivation of hydrobionts and plants is integrated [1, 2, and 3]. This innovative technology possess different advantages like: effective usage of water, decrease the volume of wastes coming from aquaculture, diversify

the products from aquaculture sector (production of plants and fish), received production is ecological, soil borne pathogens are missing, year round production could be received, could be organized at places where conventional agriculture is not possible (like example roof top aquaponic farm), could decrease the transport costs as well as pollution coming from transportation of products because it can be organized directly at the cities [2, 4, 5, and 6]. Together with different advantages like an intensive aquaculture technology, aquaponics also has different disadvantages connected with high stocking densities which are used for hydrobionts cultivation: increased stress, higher susceptibility to diseases, reduced growth, and feed intake in cultivated hydrobionts. One possible solution in the past to answering of mentioned challenges was the treatment of water species with antibiotics [7]. The aquaponics is sustainable and ecologically friendly technology which is excluding the application of these substances. The application of antibiotics in aquponics could lead to the transfer of drug resistance from fish bacterial diseases to human bacterial diseases and contributing to most significant healthcare problems further [8].

One alternative of antibiotics for treatment of fish and plants in aquaponics could be application of different plant's extracts. Different studies showed that extract of different medicinal plants could increase the growth of hydrobionts [9, 10], stimulate their immune system [11, 12, and 13], and decrease the stress in hydrobionts [14].

Yarrow (*Achillea millefolium*) is medicinal plant used in Bulgarian traditional medicine to improve the condition of the digestive tract and could positively affect digestibility and assimilation of feed nutrients, as well as physiological condition in human and animal organisms. The studies connected with the effect of this herb in fish diets are rare [15].

The aim of current study was to find the effect of feed, supplemented with yarrow (*Achillea millefolium*) on: growth performance, blood parameters and meet quality in rainbow trout (*Oncorhynchus mykiss* W.) as well as on growth of heads and roots in lettuce (*Lactuca sativa*), raised in the aquaponic recirculation system.

2. Materials and Methods

2.1 The experimental fish

The rainbow trout's in good health condition were chosen and transported from trout farm Bukovetz (Tvyrdica) to the experimental aquaculture base (Faculty of Agriculture, Trakia University, Stara Zagora). The fish were split into the following experimental groups:

- Experimental group (EF) was the fish were fed with feed supplemented with yarrow (*Achillea millefolium*) extract.

- Control group (CF) was the fish were fed with feed without the addition of supplement.

The experiment was carried out in two replications for each variant. The average initial weight of trout's from both repetitions of experimental groups were: EF - 109.8 ± 2.10 g, and CF - 114.4 ± 2.72 g.

The fish was cultivated in experimental tanks at 33 specimens/m^3 stocking density. The trial continued for 60 days.

2.2 Recirculation aquaculture system

The aquaponic recirculation system consisted of 10 cultivation tanks. The cleaning block of the system was consisted of one mechanical filter (settling tank) and moving bed biofilter. Two heaters and pump assuring recirculation of water were located in the sump. The useful volume of tanks for fish cultivation was 0.3 m^3.

Figure1. Aquaponic recirculation aquaculture system used in the current trial:
A - Aquaculture recirculation section;
B - Aquaponic section

The flow rate in recirculating aquaculture system (RAS) was maintained at 50 L/min^1. Every day the bottoms of fish tanks as well as the bottoms of filter's units were cleaned by opening the valve located at the bottoms of tanks. The water loses connected with cleaning process and evaporation were 10% of total volume of RAS. This quantity of water was refilled daily with clean water.

2.3 Experimental feed

The experimental fish were fed with a commercial extruded feed produced by the "Aqua-Garant" with the extruded pellets sized 6 mm. The content of commercial trout's feed, used in a current trial could be seen in Table 1.

Table 1. Nutritional content of commercial feed, used in the trial

Ingredients	Unit	Content
Crude protein	%	45
Fibers	%	2
Fat	%	16
Phosphorus	%	1
Vit.A	IU/kg^{-1} x 1000	10
Vit.D$_3$	IU/kg^{-1}	1500
Vit. E	mg	200

Yarrow extract was added to the granules at a concentration of 15 gr/kg fish feed. The sunflower oil at the quantity of 5 mL was also added to every 100 g of experimental feed (EF) and the components were very well mixed. The same amount of sunflower oil was added to the control feed (CF) and the pellets and oil were also mixed. Afterward, both tested feeds were left for 12 hours at an air temperature of 20 ^{0}C and were used for the feeding of experimental fish. The daily diet was maintained at 1.8% of the fish biomass. The feed was supplied manually three times per day.

2.4 Investigated parameters

2.4.1 Growth parameters in experimental fish

The mortality cases in experimental tanks were recorded daily during the trial and survival (%) was calculated using the following formula:

$$\text{Survival (\%)} = \\ = (\text{final number of fish} / \text{initial number of fish}) \times 100 \quad (1)$$

The experimental fish were weighed at the technical balance with accuracy 0.01 g at the start of the trial. The average final weight (g) was measured at the end of the experiment and specific growth rate (SGR) (%.day^{-1}) and FCR were calculated with the following equations:

$$\text{SGR (\% body wt gain/day)} = \\ = [\text{Ln final weight (g)} - \text{Ln initial weight(g)}] / \\ / \text{number of days} \times 100 \quad (2)$$

$$\text{FCR} = \text{Fed feed (g)} / \text{Weight gain of fish (g)} \quad (3)$$

2.4.2 Blood biochemical parameters in experimental fish

The blood was taken from the hearts of the examined fish (6 specimens per variant) with disposable sterile plastic syringes (3 mL) with a needle**.** Heparin sodium (1%) was used as an anticoagulant. The blood was centrifuged at 3000 rpm for separating the plasma. Afterward the biochemical parameters (glucose (GLU) (mmol/L), urea (UREA) (mmol/L), total protein (TP) (g/L), albumin (ALB) (g/L), alanine aminotransferase (ALAT) (U/L), aspartate aminotransferase (ASAT) (U/L^1), alkaline phosphatase (ALP), the content of calcium (Ca) (mmol/L), phosphorus (P) (mmol/L), magnesium (Mg) (mmol/L), triglyceride (TG) (mmol/L^{-1}) and cholesterol (CHO) (mmol/L^{-1}) in blood plasma, were examined by the colorimetric method with blood analyzer (Mindray SC - 120).

2.4.3 Meat quality in experimental fish

The fish fillets from the back side of 6 fish specimens per variant were obtained and homogenized at the end of the trial. The received muscles homogenates were used for analysis of moisture, crude protein, fat and ash in a Central research laboratory (Faculty of Agriculture, Trakia University) according to the following methods:

- Moisture (%) and dry matter (%) - Bulgarian State Standard (BSS)11374-86;
- Crude protein content, % (BSS-ISO 5983, the Kjeldahl method on Kjeltec 8400, FOSS, Sweden);
- Fat content, % (BSS-ISO 6492, Soxhlet extraction method, using Soxtec 2050, FOSS, Sweden);
- Crude ash content, % (BSS 11374-86).

2.4.4 Statistical analysis of data

The data were analyzed statistically with ANOVA single factor STATISTICA 6.0 software (StatSoft Inc., 2002).

3. Results and Discussion

3.1 Growth parameters in experimental fish

The survival of fish was 100% in both tested variants. The average values of initial weight in rainbow trout from both groups, experimental and control were very close and the difference between them was not significant ($P \geq 0.05$) (Table 2). The fish from the experimental group, fed with a supplement of yarrow had with 11.1% higher average final weight compared to the parameter's value in trout's from the control variant ($P < 0.001$). The average value of SGR in rainbow trout from the experimental variant was higher with 49.3% compared to the value of SGR in fish from CF group (Table 2). The average value of FCR found for trout's from experimental variant was lower with 36.7% compared with this, calculated for fish from control group (Table 2).

Table 2. Growth parameters in rainbow trout (*Oncorhynchus mykiss*)

Growth parameters	n	$\bar{x} \pm$ SD	
		CF	EF
Initial weight (g)	20	114.4 ± 12.18	109.8 ± 2.1
Final weight (g)	20	155.7 ± 17.82	185.2 ± 3.54*
SGR (% body wt gain/day)	2	0.50 ± 0.27	0.77 ± 0.07
FCR	2	2.42	1.53

*Shows significant statistical differences ($p < 0.05$).

The received results for growth parameters, SGR and FCR are in confirmation of studies made from Bahabadi *et al.*, [15] who found better growth, SGR and favorable FCR in trout fed with yarrow supplement, compared with the values in these parameters found for fish from the control group. Growth promoting effect was found in broilers, when their feed was supplemented with yarrow *(Achillea millefolium)* [16].

3.2 Blood biochemical parameters in experimental fish

The blood biochemical parameters in experimental fish were not significantly affected by supplementation of yarrow (*Achillea millefolium*) extract ($P \geq 0.05$) with exception of aspartate aminotransferase, alkaline phosphatase and cholesterol levels (Table 3).

The average values of ASAT, ALP, and CHO in trout's from the control variant were higher respectively with 27.4%, 57.2% and 24.9%, compared with average values in these blood parameters found out for fish from control variant ($P < 0.05$). The hepatoprotective effect (the liver enzymes and cholesterol level) was found for the trout's fed with supplemented feed in the current study. The received from us results were controversial to study made from Bahabadi *et al.*, [15], who stated that a significant increase in AST, ALP and total complement activity was observed in the fish fed with 1% yarrow extract diet. Elabd *et al.*, [17] found the improvement of aspartate aminotransferase (AST) alanine transaminase (ALT) activities, glucose and cortisol concentrations when the diet for the feeding of yellow perch was supplemented with other medicinal plants - *Astragalus membranaceus* and *Glycyrrhiza glabra*. The decreased triglyceride and cholesterol level found in current study is in confirmation with study made from Bahabadi *et al.*, [15] where triglyceride and cholesterol levels was significantly decreased in the fish fed with diets containing 0.5% and 1% yarrow extract.

3.3 Meat quality in experimental fish

The meat quality parameters in experimental fish were not significantly affected by the supplementation of feed with yarrow. The content of crude protein was higher in trout's from EF with 1.16% compared with the average value of this parameter found for fish from CF, but the difference was not significant ($P \geq 0.05$) (Table 4). The fat content was lower in trout fed with feed supplemented with yarrow with 9.16% compared with the average value found of this parameter found for trout from control variant, but the difference was not proven statistically ($P \geq 0.05$) (Table 4).

The studies connected with the influence of yarrow on meat quality in fish are missing by our understanding. The researches with other animals showed that yarrow as animal feed additives did not affect the carcass treats in broilers [16].

3.4 Yield of cultivated in aquaponics lettuce

The weight of head and roots in lettuce cultivated in aquaponic system were respectively 95.4 ± 3.06 g and 26.9 ± 0.82 g in the end of trial. The weight of head in salad in current study was similar to other study made

Table 3. Blood parameters in rainbow trout (*O.mykiss*) during the trial

Blood parameters	Unit	n	$\bar{x} \pm SD$	
			CF	EF
GLU	mmol/L	6	3.95 ± 0.45	2.83 ± 0.48
UREA	mmol/L	6	1.53 ± 0.23	1.37 ± 0.18
CREA	µmol/L	6	21.36 ± 5.55	21.75 ± 4.53
TP	g/L	6	55.92 ± 2.77	53.06 ± 6.54
ALB	g/L	6	33.35 ± 1.53	34.41 ± 3.33
ASAT	U/L	6	78.27 ± 27.3	56.36 ± 4.41*
ALAT	U/L	6	25.08 ± 9.02	28.22 ± 13.15
ALP	U/L	6	316.54 ± 55.2	135.44 ± 40.8*
Ca	mmol/L	6	3 ± 0.19	2.71 ± 0.34
P	mmol/L	6	4.48 ± 0.52	3.96 ± 0.25
Mg	mmol/L	6	1.16 ± 0.06	1.37 ± 0.11
TG	mmol/L	6	1.54 ± 0.07	1.47 ± 0.08
CHO	mmol/L	6	7.93 ± 0.66	5.95 ± 0.50*

*Shows significant statistical differences ($P < 0.05$).

Table 4. Meat quality parameters in fish during the trial

Meat quality parameters	n	$\bar{x} \pm SD$	
		CF	EF
Moisture	6	77.26 ±0.10	77.22 ±0.16
Dry matter	6	22.73 ±0.10	22.77 ±0.16
Crude protein	6	18.61 ±0.18	18.83 ±0.06
Fat	6	2.51 ± 0.09	2.28 ± 0.09
Ash	6	1.60 ± 0.01	1.65 ± 0.03

*Shows significant statistical differences ($p < 0.05$).

in aquaponics where Delaide *et al.*, [18] received 80.55 g for shoot and significantly lower average fresh weight of plants roots 5.8 g, which difference could be result from different availability of nutrients in aquaponics for plants in two studies. The received from aquaponic system products are organic, ecological and healthy food for people [19, 20].

4. Conclusions

- The extract from yarrow (*Achillea millefolium*) added to feed for the feeding of rainbow trout (*O. mykiss*) increases the growth of fish and significantly affect the level aspartate aminotransferase, alkaline phosphatase and cholesterol, but did not affect the meat quality parameters of experimental fish.

- The extract from yarrow used as a supplement in the feeding of rainbow trout (*O. mykiss*) improve feed conversion ratio (FCR) in experimental fish.

- The weight of head in lettuce cultivated in aquaponic system were similar to this found in other studies but the fresh weight of lettuce roots was lower in current trial.

-Yarrow extract used as a supplement improve feeding and physiological condition in fish without significantly affect the productivity in aquaponic aquaculture.

5. References

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