The effect of sweet flag (*Acorus calamus* L.) supplemented diet on growth performance, biochemical blood parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.) and growth of lettuce (*Lactuca sativa* L.) cultivated in aquaponic recirculation system

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Abstract. The aim of current study was to find the effect of feed, supplemented with sweet flag (*Acorus calamus* L.) 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* L.), raised in the aquaponic recirculation system. Ten specimens from the rainbow trout with an average weight of 114.45±12.18 g (control, C) and 108.85±21.24 g (experimental, A. c.) in good health condition were placed in each tank of aquaponics recirculation system and cultivated for 60 days. At the end of the experiment were calculated: average final weight, specific growth rates, feed conversion ratio, meat quality and blood biochemical parameters. 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 fish fed with feed supplemented with *A. calamus* extract showed higher final weight with 8.84%, compared to the values of this parameter of trouts from the control group at the end of the experiment ($p \leq 0.01$). The average values of ALB and ALAT were respectively with 26.84% and 52.26% higher in the control group, compared to the value of the same parameter of trouts fed with the *A. calamus* extract supplement ($p \leq 0.05$). The content of crude protein was higher in trouts from experimental group with 1.1% compared with the average value of this parameter found for fish from control group ($p \geq 0.05$). 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. Sweet flag extract used as a supplement improve feeding and physiological condition in fish without significantly affect the productivity in aquaponic aquaculture.

Key Words: aquaponic, hydrobiont, growth, fish, sweet flag.

Introduction. The continuous growth of the earth's population leads to pollution of soil, air and water. It is necessary to ensure organic production and healthy food for humans (Valkova et al 2015). Aquaponics is a new technology based on the combined cultivation of fish and plants (Velichkova et al 2019b). The advantages of this innovative technology are: efficient use of water, reduction of the volume of waste from aquaculture, diverse products from the aquaculture sector (production of plants and fish), the resulting production is ecological, no soil transmissible pathogens, year-round production can be obtained, it can be arranged in places where conventional agriculture is not possible (as an example aquaponic roof truss), it can reduce transport costs as well as pollution coming from transport of products because it can be organized directly into cities (Sirakov et al 2019b). Along with various advantages, such as intensive aquaculture technology, aquaponics also has various disadvantages associated with high density of cultivation, which is used for hydrobionts cultivation: greater susceptibility to diseases, increased stress, reduced growth and food intake in cultivated aquatic organisms.

Treatment with antibiotics has been used as a solution in the past of aquatic species farming (Sirakov et al 2018). Aquaponics precludes the use of these substances as it is sustainable and environmentally friendly technology. The use of antibiotics in aquaponics can lead to the transfer of drug resistance from fish bacterial pathogens to human bacterial pathogens and contributing to the most significant health problems. A variety of plant extracts can be applied as an alternative to antibiotics to treat fish and plants in aquaponics (Velichkova et al 2019a). Various studies have shown that the extract of various medicinal plants can increase the growth of hydrobionts (Sirakov et al 2019a), improve digestion, stimulate their immune system (Velichkova & Sirakov 2019) and reduce stress in hydrobionts (Shah & Gilani 2012). Such a medicinal plant, used since ancient times and with proven healing properties, is the sweet flag, *Acorus calamus*.

A. calamus has a number of pharmacological effects, such as the central nervous system depressant (Vengadesh Prabu et al 2009), cardiac depressant (Shah & Gilani 2012), antidiabetic (Prisilla et al 2012), antioxidant (Subathraa & Poonguzhali 2012), anti-inflammatory (Kim et al 2009), antifungal (Begum et al 2007), antibacterial (Manikandan et al 2010), insecticide (Nalamwar et al 2009). Essential oils, glycosides, alkaloids, tannins, vitamin C, starch, mucus, resin have been found in the leaves and rhizomes of the sweet flag (Subathraa & Poonguzhali 2012). It has been found that the herb has the ability to increase the secretion of gastric juice (Sharma et al 2020). This property can have a positive effect on the growth and health of fish if they consume this plant by improving the intestinal wall and increasing digestibility.

The aim of current study was to find the effect of feed, supplemented with sweet flag (*Acorus calamus* L.) 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* L.), raised in the aquaponic recirculation system.

Material and Method

Recirculation aquaculture system*.* The aquaponic recirculation system situated at the Aquaculture Base of the Faculty of Agriculture of the Trakia University consists of two parts – fish section and plant section. The system for culturing fish consisted of 10 tanks; the system was equipped with a mechanical filter (settling tank), a biological filter (moving bed biofilter) and a collecting vessel connected by Initial Waste 14.9 pump (Wilo®) to the aquaponic and fish tanks (Figure 1). Each fish tank had the volume of 300 L. The sedimentation tank was filled with plastic lamellas that reduce the water velocity and help settle dawn the waste particles. The moving bed biofilter had a filling of 50 kg plastic rings. The volume of the mechanical and biological filter and the collecting vessel is 1 m^3 (each of them). The aquaponics section consisted of 8 tanks with dimensions 1 x 0.5 x 0.25 m. A polystyrene sheet of 0.95 x 0.45 x 0.05 m was floating in each aquaponic tank. For the aeration of the cultivation vessels and the biological filter, two aerators were used. To ensure a favorable water temperature in the tanks, they are provided with two 2000 W heaters, immersed in the collecting vessel, connected to a temperature probe controller. The bottoms of fish tanks and the bottoms of filter's units were cleaned every day by opening the valve located at the bottoms of tanks. The water loses were 10% of total volume of RAS due to cleaning process and evaporation. Every day this quantity of water was refilled with clean water.

Fish and feed*.* Ten rainbow trout in good health condition with an average weight of 114.45±12.18 g (control, C) and 108.85±21.24 g (experimental, A. c.) were placed in each tank. The trial continued for 60 days. The experiment was carried out in two replications. The fish were fed with 6 mm extruded pellets "Aqua UNI", produced by "Aqua garant". In Table 1 it could be seen the content of commercial rainbow trout's feed used in the current trial.

Figure 1. Aquaponic recirculation aquaculture system used in the current trial.

Table 1

Nutritional content of commercial feed used in the trial /according to fish feed producer "Aqua garant"

Ingredients	*Unit*	*Content*
Crude protein	%	45
Fibers	%	2
Fat	%	16
Phosphorus	%	1
Vitamin A	IU kg^{-1}	10000
Vitamin D	IU kg^{-1}	1500
Vitamin E	IU kg^{-1}	200

To the fish feed of trout's from the experimental group it was added 1% *Acorus calamus* (A. c.) extract to the pellets from daily feed ratio. To every 100 g of experimental feed, sunflower oil was also added at volume of 5 mL and the components were very well mixed. Towards the control feed the same amount of sunflower oil was added and the pellets and oil were also mixed. The both tested feed were subsequently left at air temperature of 20ºC for 12 hours and were used for experimental fish feeding. The daily feed ratio was maintained at 1.8% of the total fish biomass. The feed was supplied manually three times per day. Tanks were cleaned daily and excreta were released. Light was about 12:12 h light:dark cycle throughout the day.

Hydrochemical analysis and growth parameters in experimental fish. The water temperature (ºC), pH, dissolved oxygen (mg L^{-1}) and conductivity (µS cm^{-1}) were measured daily with a portable meter HQ30D (Hach Lange), connected with a probe appropriate for each parameter.

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

Survival (%) = (final number of fish / initial number of fish) x 100

The experimental fish were weighed with a technical balance at the start of the trial. The average individual weight gain (WG) (g) and specific growth rate (SGR) (% body wt gain/day) were determined with the following equations (Zhou et al 2006):

WG (g) = Average final weight - Average initial weight

SGR (% body wt gain/day) = [Ln final weight (g) - Ln initial weight (g)] /

/ number of days x 100

Feed conversion ratio (FCR) was calculated at the end of trial with the following equation:

FCR = Fed feed (g)/ Weight gain of fish (g)

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^{-1}), urea (UREA) (mmol L^{-1}), creatinine (CREA) (µmol L^{-1}), total protein (TP) (g L^{-1}), albumin (ALB) (g L^{-1}), alanine aminotransferase (ALAT) (U L^{-1}), aspartate aminotransferase (ASAT) (U L^{-1}), the content of calcium (Ca) (mmol L^{-1}), phosphorus (P) (mmol L^{-1}), magnesium (Mg) (mmol L^{-1}), triglyceride (TG) (mmol L^{-1}) and cholesterol (mmol L^{-1}) in blood plasma were examined by the colorimetric method with blood analyzer (Mindray SC - 120).

Meat quality in experimental fish. At the end of the trial the fish fillets from the back side of 6 fish specimens per variant were obtained and homogenized. Analysis of moisture, crude protein, fat and ash were done from the received muscles homogenates 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).

Experimental lettuce. Forty lettuces (Zhalta krasavica variety) were chosen and transported from greenhouse situated in Plovdiv to aquaculture base in Stara Zagora at the stage of 3 leaf formation. The plants were transferred on specialized substrate (Grodan®) with the size 5 cm and afterwards the lettuce plants were planted in hydroponic pots and placed to experimental systems.

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

Results and Discussion

Hydrochemical analysis. One of the most important indicators for the optimal development of the cultivated species is the temperature of the water. During the experiments its values were 16.8-17.9°C in the control and experimental tanks, which are within the optimal values for trout breeding (Zaykov & Staykov 2013) (Figure 2).

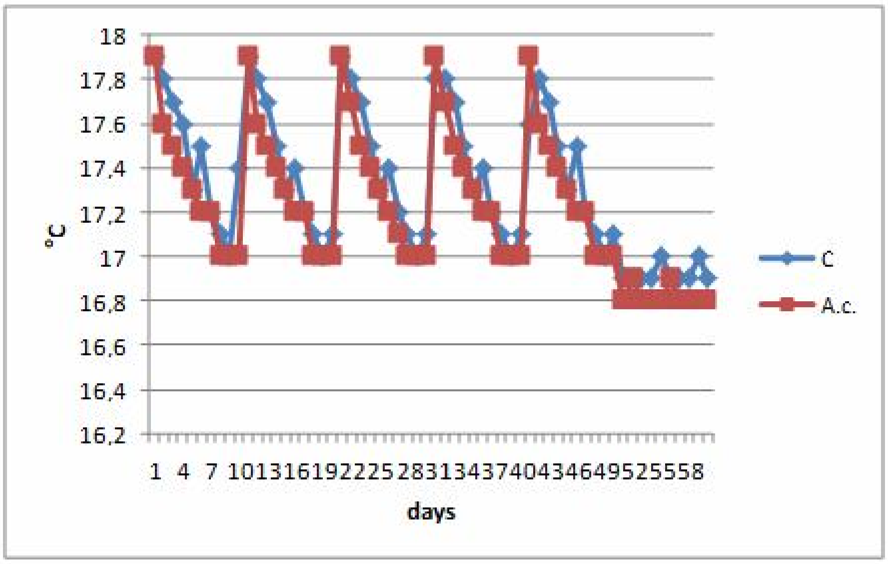

Figure 2. Water temperature in control (C) and experimental (A.c.) tanks.

The dissolved oxygen during the trial varies between 7.35 and 8.32 mg L^{-1} (Figure 3). The values of this parameter during the period were higher with 4.13% in the experimental tanks compared to those of the control tanks and the difference was statistically significant ($p \leq 0.001$). This higher dissolved oxygen level may be due to influence of plant extract supplement in feed for fish.

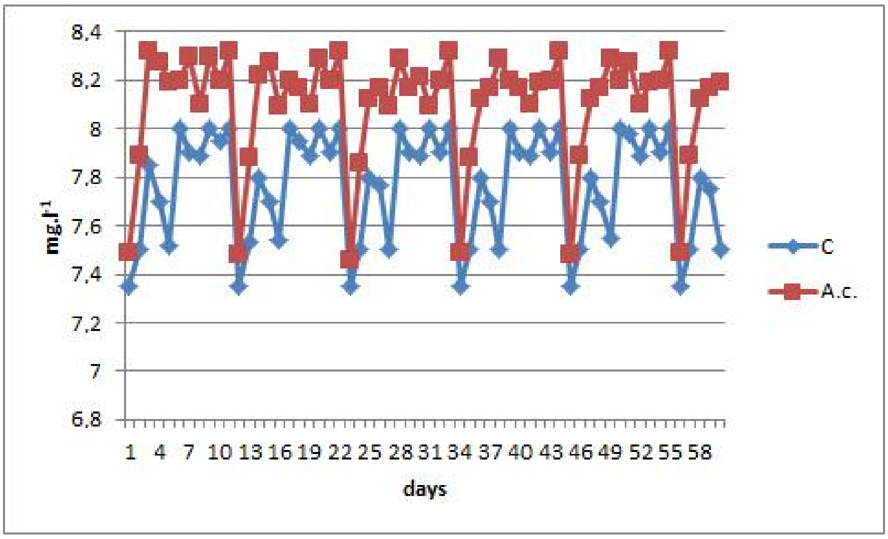

Figure 3. Dissolved oxygen in control (C) and experimental (A.c.) tanks.

The pH values of the water in the recirculation system varied between 7.4 and 8.11, which are slightly alkaline (Figure 4). It is very important that pH levels to not be below 6.5 and above 8.5, because young fish are extremely sensitive to this indicator (Sirakov et al 2019b). The better pH value (7.4) was observed in the experimental variant ($p \leq 0.001$) compared to control, which was probably due to the action of the plant extract.

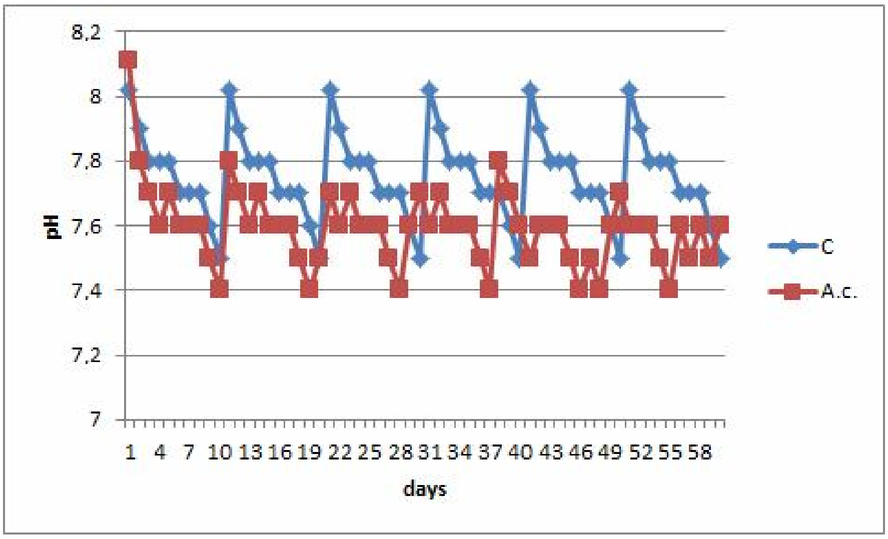

Figure 4. pH in control (C) and experimental (A.c.) tanks.

The electrical conductivity values of water ranged from 263 to 269 μS cm^{-1} (Figure 5). The values of this parameter in the experimental variant were 1.1% higher than those of the control and the difference was statistically significant ($p \leq 0.001$).

The hydrochemical data analysis showed that it was optimal for the farmed species during the trial. Three times per day experimental and control tanks were cleaned, with addition of fresh water in an amount of 10% from the total recirculation system volume. The mechanical filter and especially the biofilter was of major significance to maintain the optimum water chemical parameters during the experiment.

This in turn led to good results with respect to survival, weight gain and feed conversion ratio in experimental fish.

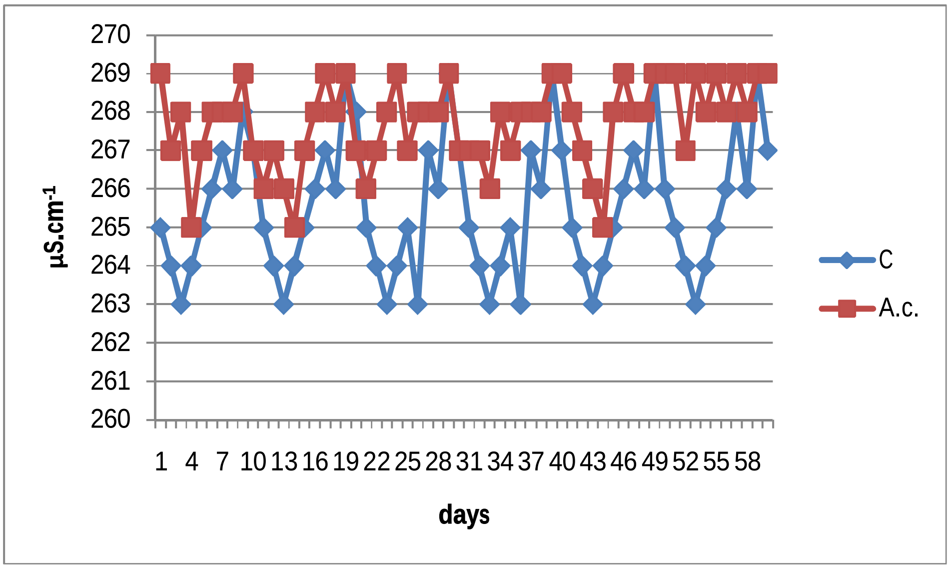

Figure 5. Electric conductivity in control (C) and experimental (A.c.) tanks.

Growth parameters in experimental fish. The survival of fish was 100% in both tested variants (Table 2). The average initial live weight of rainbow trout from control and experimental variants was, respectively 114.45±12.18 g and 108.85±21.24 g, and the differences were not statistically significant ($p > 0.05$) (Table 2). The fish fed with feed supplemented with *A. calamus* extract showed higher final weight with 8.84%, compared to the values of this parameter of trouts from the control group at the end of the experiment, and the differences was statistically significant ($p \leq 0.01$).

The average value of SGR in rainbow trout from the experimental variant was higher with 33.33% compared to the value of SGR in fish from control group (Table 2). The average value of FCR found for trouts from experimental variant was lower with 32.64% compared of FCR average value, calculated for fish from control group (Table 2). In the current trial, the values of growth parameters (final weight, average individual weight gain and SGR) and feed conversion ratio (FCR) in fish from the experimental group feed supplemented with *A. calamus* extract were higher than those found for the trout from the control group. These results are in confirmation of results, received from different authors studying feed with herbs extract supplementation and their effect of growth and feed efficiency (FE) (Zhang et al 2014; Sirakov et al 2019b).

Table 2

Growth performance of rainbow trout in control and experimental tanks

Parameter	*n*	*C*	*A.c.*
		$\bar{x} \pm SD$	$\bar{x} \pm SD$
Initial body weight, g	20	114.45 ± 12.18	108.85±21.24
Final body weight, g	20	155.70 ± 17.82**	170.8±31.63**
Survival rate, %		100	100
SGR % per day		0.50 ± 0.27	0.75±0.07
Average individual weight gain, g	20	41.25 ± 0.12	61.95±8.9
FCR		2.42	1.63

** $p \leq 0.01$.

Blood biochemical parameters in experimental fish. The creatinine level was 9.14% higher in the control group, compared to the value of the same parameter of trouts fed with the *A. calamus* extract supplement ($p \geq 0.05$) (Table 3). The average values of ALB and ALAT were respectively with 26.84% and 52.26% higher in the control group, compared to the values of the same parameters of trouts fed with the *A. calamus* extract supplement, and the difference was statistically significant ($p \leq 0.05$). The average values of ASAT and ALP in trouts from the control variant were higher respectively with 37.15% and 1.54%, compared with average values in these blood parameters found out for fish from control variant. The hepatoprotective effect was found for the trouts fed with supplemented feed in this study. Elabd et al (2006) found the improvement of aspartate aminotransferase (ASAT), alanine transaminase (ALAT) activities and glucose concentrations on the feeding of yellow perch with supplemented medicinal plants diet. Higher average values for Ca (0.84%), P (14.14%) and Mg (0.85%) were found for the experimental fish, compared with the ones of fish in control variant.

Table 3
Biochemical blood parameters of rainbow trout in control (C) and experimental (A.c.)

Blood parameters/groups	*n*	*C*	*A.c.*
GLU	6	3.47±0.93	3.61±1.28
UREA	6	1.35±0.27	1.32±0.36
CREA	6	37.2±13.51	33.8±9.41
TP	6	50.1±9.32	46.0±3.68
ALB	6	32.04±7.34*	23.44±6.13*
ASAT	6	101.83±11.1	64.0±6.6
ALAT	6	36.66±4.80*	17.5±1.54*
ALP	6	162.0±13.9	159.5±8.9
CA	6	2.37±1.3	2.39±0.94
P	6	3.40±1.62	3.96±0.91
Mg	6	1.16±0.07	1.17±0.21
TG	6	1.56±0.36	1.50±0.31
CHOL	6	6.23±1.71	6.71±1.46

*$p \leq 0.05$. Note: Glu = glucose, Crea = creatinine, TP = total protein, Alb = albumin, ASAT = aspartate aminotransferase, ALAT = alanine transaminase, ALP = alkaline phosphatase, Ca = calcium, P = phosphorus, Mg = manganesium, TG = triglycerides, CHOL = cholesterol.

Meat quality in experimental fish. The content of crude protein was higher in trouts from experimental group with 1.1% compared with the average value of this parameter found for fish from control group, but the difference was not significant ($p \geq 0.05$) (Table 4). The fat content was lower in trout fed with feed supplemented with sweet flag with 0.98% 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 ash content of the trout fillets in the experimental group was higher by 8.05% compared to the fish in the control and the difference was statistically significant ($p < 0.001$) (Table 4).

Table 4
Chemical composition of meat from rainbow trout (*O. mykiss*) in control (C) and experimental groups (A.c.) in tanks (%)

Group	*n*	*Moisture*	*Dry matter*	*Crude protein*	*Fat*	*Ash*
C	6	77.26±0.26	22.73±0.26	18.61±0.44	2.51±0.22	1.60±0.02***
A.c.	6	76.99±0.55	23.00±0.55	18.81±0.20	2.45±0.42	1.74±0.03***

***$p \leq 0.001$.

Yield of lettuce grown in aquaponics. 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. Delaide et al (2016) in aquaponics similar study received 80.55 g for weight of head and significantly lower average fresh weight of plants roots 5.8 g. This could be resulted from different availability of nutrients in aquaponics for plants. The cultivation of plants and hydrobionts in an aquaponics system leads to organic, ecological and healthy food production for people (Stoyanova et al 2019).

Conclusions. The extract from sweet flag (*Acorus calamus*) added to feed for the feeding of rainbow trout (*O. mykiss*) led to increases of fish growth and improved feed conversion ratio (FCR) in experimental fish. The hepatoprotective effect was found at the trouts fed with sweet flag supplemented feed and significantly affected the albumin level and alanine transaminase. Sweet flag extract used as a supplement improved feeding and physiological condition in fish without significantly affecting the productivity in aquaponic aquaculture. Future studies with different concentrations and different extracts of *Acorus calamus* are needed to be tested in aquaculture.

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