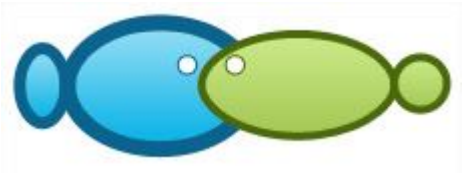

Growth efficiency, biochemical blood parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.) fed with supplement of white yarrow extract (*Achillea millefolium* L.)

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Abstract. In recent years, more and more experiments have been made with the addition of various plant extracts to fish feed. The purpose of this study was to examine the influence of white yarrow (*Achillea millefolium*) extract added to rainbow trout (*Oncorhynchus mykiss*) feed on growth, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol). In order to achieve the purpose, in a recirculating system in the Aquaculture Base of the Faculty of Agriculture - Trakia University, Stara Zagora, experiments were conducted with a control group (without supplements to feed C) and experimental A.m (supplemented with 1666 mg kg^{-1} white yarrow extract A.m), each with two repetitions. Thirty specimens of rainbow trout with average fish weight of 42.55±7.48 g (C) and 42.46±7.56 g (A.m) in good health were placed in each tank and cultivated for 60 days. At the end of the experiment, average final weight, specific growth rates, feed conversion factor, meat quality and blood parameters were established. At the end of the experiment, the average live weight of fish fed with white yarrow extract was 21.34% higher than the value of the same indicator of the trout in the control group ($p < 0.05$). ASAT blood biochemical parameter in the control variant was with 29.88% higher compared to the same value in the experimental group ($p > 0.05$). The white yarrow extract added to the feed resulted in a 21.59% reduction in the lipid content of the rainbow trout fillets compared to the control group and the differences was statistically proven ($p < 0.01$). Rainbow trout, fed with a nutritional supplement from white yarrow extract, had better indicators of growth parameters and meat quality.
Key Words: biochemical blood parameters, growth, meat quality, rainbow trout, white yarrow.

Introduction. Various plants, as well as extracts thereof, can be used as a supplement in human and animal food. In recent years, increasingly experiments have been made with the addition of various plant extracts to fish feed in order to improve the absorption coefficient, to increase meat quality and survival rates (Gabor et al 2011; Sirakov & Velichkova 2014; Velichkova et al 2018). Experiments also have been made to improve the different blood parameters. Plant extracts have several advantages: they contain active substances that are of natural origin and not synthetic, making them safe for fish and for environment; they are derived relatively easily from plants by many known methods which are not complex; very rarely they cause undesirable side effects; overdosing with them is difficult (Velichkova et al 2016; Sirakov & Velichkova 2018). There is still not much research on the impact of plant supplements and plant extracts on different fish species, their growth and health. Some herbal extracts can be used as an alternative to antibiotics in aquaculture (Sirakov et al 2015; Ali et al 2018). Many plant species are known to possess pharmacological properties such as antimicrobial, anti-inflammatory, anti-tumour, anti-proliferative properties (El-Dakar et al 2015). The advantage of herbal therapy is that it does not burden the body of the fish (Velichkova & Sirakov 2013). However, few are the studies that have assessed the effect of phytoadditives on growth indicators and feed conversion parameters for different farmed fish species. One of the applications of white yarrow, *Achillea millefolium* L. in traditional

medicine is to increase bile secretion and in inflammation of the gastrointestinal tract (Benedek et al 2008). The above-ground parts of the white yarrow plant, stems and flowers are traditionally used as a "bitter" tonic to improve appetite and digestion (Simic et al 2002; Candan et al 2003). There is no date of the use of white yarrow or extract thereof in the cultivation of hydrobionts. The purpose of this study is to establish the growth rate, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol) of rainbow trout (*Oncorhynchus mykiss*), fed with white yarrow extract (*Achillea millefolium*).

Material and Method

Experimental fish and nutrition. One hundred and twenty (120) specimens of rainbow trout with an average weight of 42.55±7.48 g (control C) and 42.46±7.56 g (experimental A.m) in good health were placed in tanks and cultivated for 60 days, January-March, 2018. Concrete tanks have an effective water volume of 0.8 m^3, which is part of a recirculation system. The fish from the control group C (without supplements) and the experimental A.m (supplemented with *Achillea millefolium* extract), each with two repetitions (30 specimens per repetition), were placed in a recirculating system at the Aquaculture Base of the Faculty of Agriculture of the Trakia University. The fish are fed with 6 mm extruded pellets "Aqua UNI" produced by "Aqua guarant". To the fish feed of the trout from the experimental group 1666 mg kg^{-1} of white yarrow extract was added as well as 5 mL of sunflower oil for every 100 g of pellets. Rainbow trout from the control group are fed with granules coated only with the same amount of sunflower oil. The nutrient content in the feed of the two groups consists of: 45% crude protein, 16% crude lipids, 2% crude fibre, 1% P, 18.5 MJ kg^{-1} ME, 10000 IU kg^{-1} Vitamin A, 1500 IU kg^{-1} Vitamin D3, 200 mg kg^{-1} Vitamin E. The daily ration of the fish studied is 1.8% of their weight and are fed three times a day (8 a.m., 12 p.m, 16 p.m). Tanks are cleaned daily and excreta are released. The light intensity is about 12:12 hours light:dark cycle for a day.

Fish growth parameters. The average individual fish weight (g) is calculated at the beginning, middle and end of the experiment to establish the effect of white yarrow extract on weight gain and feed conversion ratio (FCR) of the rainbow trout grown in the recirculation system. At the end of the experiment, weight gain (g), survival rate (%) and FCR were determined.

The growth calculations were performed according to the following formulas:

- Specific Growth Rate (SGR) (Zhou et al 2006):

$$SGR = \frac{LnWf - LnWi}{n} \times 100$$

where: SGR = specific growth rate, %;
Wi = initial weight, g;
Wf = final weight, g;
n = number of days.

- Feed conversion ratio (FCR):

$$FCR = \frac{\text{Feed given}}{\text{Fish weight gain}}$$

where: FCR = feed conversion ratio;
feed given, g;
fish weight gain, g.

Hydrochemical parameters. The oxygen content (mg L^{-1}), pH, water temperature (°C) and electrical conductivity (µS cm^{-1}) were measured daily with a portable measuring instrument (HQ30D). Other parameters for water quality, ammonia (mg L^{-1}) and phosphates (mg L^{-1}) were observed weekly at Ecolab Faculty of Agriculture, Trakia University, Stara Zagora.

Chemical analyses of meat samples. Samples of rainbow trout muscles were determined on Perkin Elmer Atomic Absorption Spectrometer (AAS) "A Analyst 800". The crude protein content (%) was calculated by converting the nitrogen content quantified by the Kjeldahl method using an automated Kjeldahl system (Kjeltec 8400, FOSS, Sweden). The lipid content (%) was determined by the Soxhlet method using an automated system (Soxtec 2050, FOSS, Sweden). The ash content (%) was investigated by burning in a muffle furnace (MLW, Germany) at 550°C for 8 hours. Samples were brought to room temperature and weighed.

Biochemical blood analyses. Blood was taken directly with sterile disposable syringes (3 mL) with a needle from the heart of the fish being examined. Sodium heparin (1%) was used as anticoagulant. Blood samples were immediately transmitted and analysed in a haematological laboratory (NCPTC - Trakia University) and reported in a Mindray BC-120 haematology analyser. The following biochemical blood parameters were examined: glucose (mmol L^{-1}), urea (mmol L^{-1}), creatinine (µmol L^{-1}), total protein (g L^{-1}), albumin (g L^{-1}), aspartate aminotransferase (ASAT) (U L^{-1}), alanine transaminase (ALAT) (U L^{-1}), calcium (mmol L^{-1}), phosphorus (mmol L^{-1}), magnesium (mmol L^{-1}), triglycerides (mmol L^{-1}) and cholesterol (mmol L^{-1}).

Statistical analysis. Data obtained from the study were statistically analysed with an ANOVA single factor (MS Office, 2010).

Results and Discussion. Water temperature is one of the most important indicators for the optimal development of cultivated species. Its values were 16.8-17.9°C in the control and experimental tanks, which are within the optimal values for trout breeding (Kurdomanov et al 2019) (Figure 1).

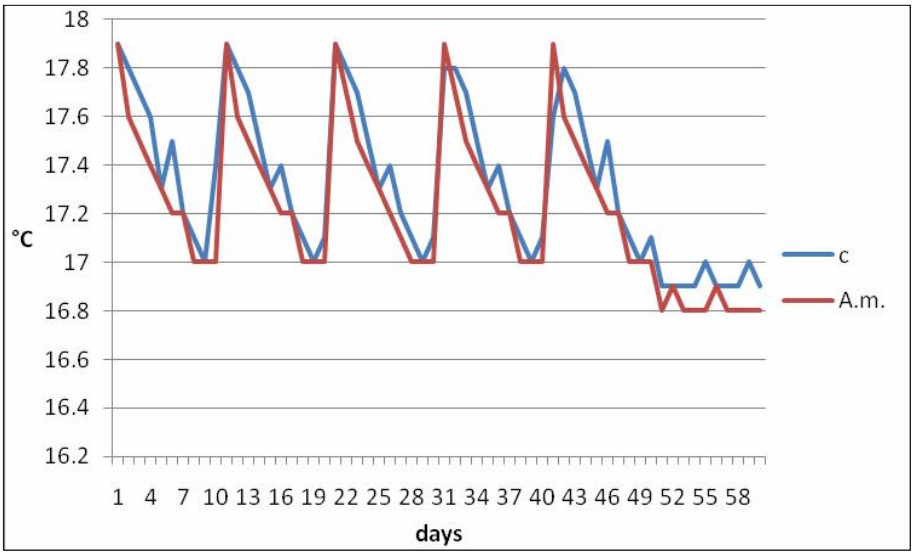

Figure 1. Water temperature in control (C) and experimental (A.m) tanks.

The dissolved oxygen during the experiment varies between 7.35 and 8.32 mg L^{-1} (Figure 2). The values of these parameters during the period were higher with 3.48% in the experimental tanks compared to those of the control tanks.

It is very important that pH levels are not below 6.5 and above 8.5, because young fish are extremely sensitive to this indicator (Sirakov et al 2019b). The pH values of the water in the recirculation system vary between 8.02 and 7.3, which are slightly alkaline (Figure 3).

The electrical conductivity values of water ranged from 263 to 269 µS cm^{-1} (Figure 4). The values of this parameter in the experimental variant were 0.73% higher than those of the control.

Analysis of the hydro chemical data shows that during the experiment their values were optimal for the cultivated species. The tanks were cleaned three times a day by

adding fresh water at a rate of 10% of the total volume of the recirculation system. Mechanical and biological filters are of great importance to maintain optimal water chemical parameters during the experiment.

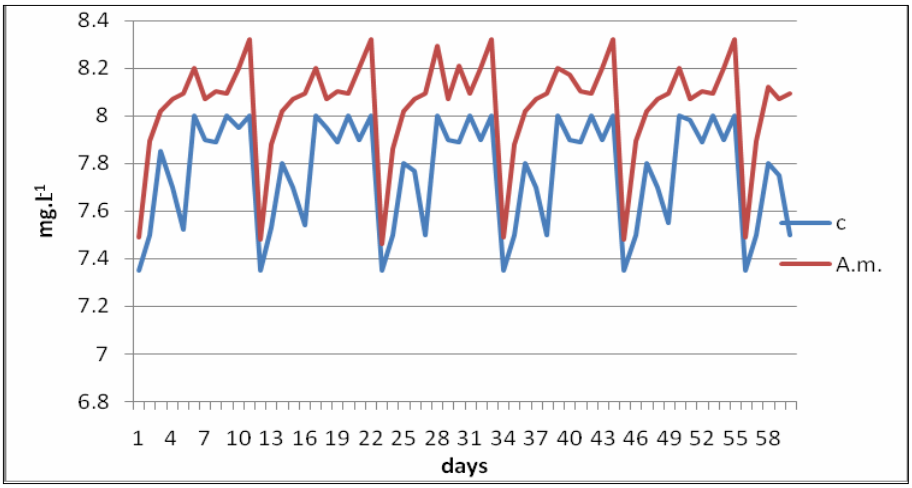

Figure 2. Dissolved oxygen in control (C) and experimental (A.m) tanks.

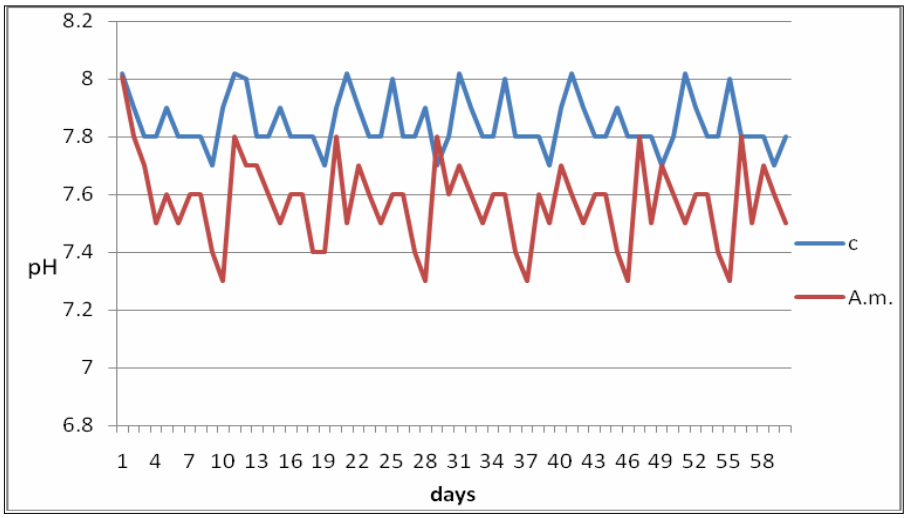

Figure 3. pH in control (C) and experimental (A.m) tanks.

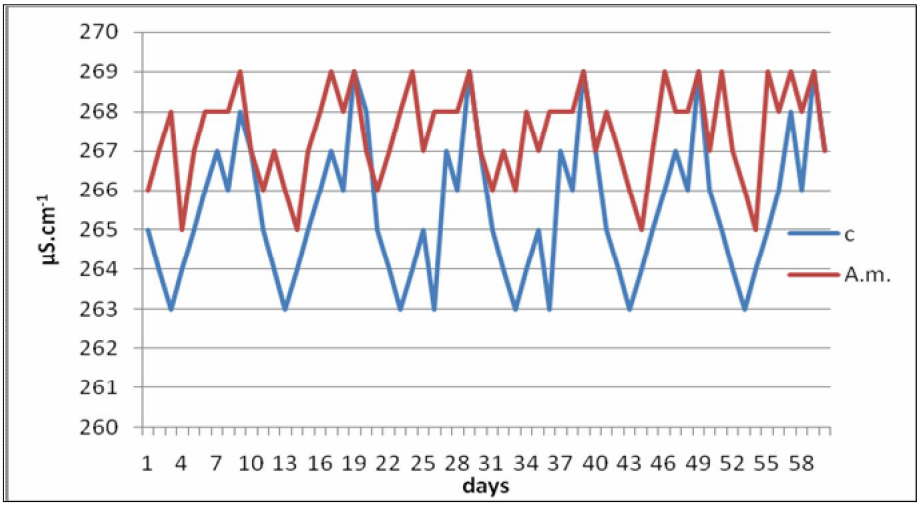

Figure 4. Electric conductivity in control (C) and experimental (A.m) tanks.

The concentration of ammonia over a period of 60 days was averaged 0.78 mg L^{-1} in the water of the experimental tanks, and 0.55 mg L^{-1} in the control tanks, but the differences was not statistically proven ($p > 0.05$). Phosphorus averaged 0.8 mg $L^{-1}$and 1.34 mg $L^{-1}$for control and experimental tanks, respectively, but the differences were not statistically proven ($p > 0.05$) for both groups during the experiment (Table 1).

Table 1

Concentration of ammonia and phosphorus in control (C) and experimental (A.m) tanks

Group	*n*	*Parameters (mg L^{-1})*	
		NH_4^+	*P-PO_4^-*
C	8	0.55±0.12	0.80±0.54
A.m.	8	0.78±0.29	1.34±1.15

P > 0.05 (ns).

The mean initial live weight of rainbow trout from control and experimental variants is 42.55±7.48 g and 42.46±7.56 g, respectively, and the differences are not statistically significant ($p > 0.05$) (Table 2). At the end of the experiment, the mean live weight of fish fed with white yarrow extract is 133.9±17.92 g, which is 14.51% higher compare to the value of the same indicator of the trout in the control group ($p < 0.05$) (Table 2). Survival during the experiment is 100% in fish in the experimental and control groups (Table 2). At the end of the study, the feed consumption analysis show that FCR of the trouts from the experimental group is 1.14 and with 21.91% lower than that of the fish from the control group (1.46) (Table 2). The rainbow trout growth parameters are higher in the group fed with white yarrow extract.

Table 2

Rainbow trout growth parameters in control (C) and experimental (A.m) tanks

Parameter	*n*	*C*	*A.m.*
		$\bar{x} \pm SD$	*$\bar{x} \pm SD$*
Initial body weight, g	30	42.55±7.48	42.46±7.56
Final body weight, g	30	114.46±17.03***	133.9±17.92***
Survival rate, %		100	100
SGR, % per day		1.64±0.03*	1.91±0.04*
Average individual weight gain, g	30	71.91±0.02*	91.43±0.06*
FCR		1.46	1.14

*P < 0.05; *** P < 0.001.

Biochemical blood parameters have been used as valuable tools for monitoring fish health and the normal ranges of the main biochemical parameters have not yet been defined for different species in different aquaculture conditions (Satheeshkumar et al 2011). In the present study, the glucose level is 39.36% higher in the fish from the control group, but the differences are not statistically proven ($p > 0.05$) (Table 3). The urea level is 23.02% higher in the fish from the experimental group compared to those of the control variant, but without statistically significant differences ($p > 0.05$). The creatinine level is 40% higher in the control group compared to feeding with the white yarrow extract ($p > 0.05$). The total protein level is 10.49% lower in the rainbow trout fed with supplement compared to the control, although this difference is not statistically significant ($p > 0.05$). According to Hrubec et al (2001) the protein level increases with age. Albumin transports hormones, vitamins and substances like calcium in the body (Satheeshkumar et al 2011). It also prevents fluid from leaking blood vessels and nourishes tissues. The amount of albumin in the experimental fish blood is 12.44% lower than the control group. ASAT values in the control group are 29.88% higher than in the experimental group ($p > 0.05$). ALAT values in the experimental group are 65.34% higher than the control group, and the result is not statistically significant ($p > 0.05$). ALP indicator in blood of the trout in the control group is 73.98% lower than in the experimental group, without statistically

significant difference ($p > 0.05$). Cholesterol and triglyceride levels may be affected by pollutants (Adham et al 2002; Yang & Chen 2003). Triglyceride concentrations in the serum of rainbow trout fed with supplement are 20.32% higher than in the control fish ($p > 0.05$). The cholesterol measured is 29.03% lower in the experimental variant compared to the control group but without statistically significant differences ($p > 0.05$). Changes in blood electrolytes may lead to disturbances in normal vital physiological functions of fish and its growth rate (Prasad et al 2011; Velichkova et al 2019). Calcium is a very important mineral element and is present in larger quantities than other electrolytes. It is contained in bones in combination with phosphorus in the form of calcium phosphate. Ionized calcium is very important for normal muscle excitement and blood clotting (Kulkarni 2015). In this study, the calcium levels in the blood of rainbow trout from the experimental group are 25.67% higher than the levels of the same mineral in the control group ($p > 0.05$). Phosphorus levels in the control group are 11.31% higher than in the experimental group ($p > 0.05$). Magnesium is 59.65% higher in the fish in the experimental group compared to the control group but the differences are not statistically proven ($p > 0.05$).

Table 3

Biochemical blood parameters of rainbow trout in control (C) and experimental (A.m)

Blood parameters	*n*	*C* ($\bar{x} \pm SD$)	*A. m.* ($\bar{x} \pm SD$)
Glu, mmol L^{-1}	6	4.70±0.61	2.85±1.65
Urea, mmol L^{-1}	6	1.17±0.95	1.52±0.56
Crea, µmol L^{-1}	6	10±1.20	6.0±5.69
TP, g L^{-1}	6	45.47±4.21	40.70±23.18
Alb, g L^{-1}	6	22.5±2.83	19.70±10.32
ASAT, U L^{-1}	6	153.33±147.41	107.5±86.26
ALAT, U L^{-1}	6	12.33±1.70	35.71±13.71
ALP, U L^{-1}	6	41.67±16.26	160.17±136.98
Ca, mmol L^{-1}	6	1.94±1.16	2.61±1.11
P, mmol L^{-1}	6	6.98±0.64	6.19±3.58
Mg, mmol L^{-1}	6	0.94±0.20	2.33±1.48
TG, mmol L^{-1}	6	0.98±0.84	1.23±0.46
GGT, U L^{-1}	6	2.33±1.04	1.40±1.13
CHOL, mmol L^{-1}	6	4.27±0.72	3.03±2.50

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

Adding white yarrow extract to rainbow trout diet lead to result in 2% higher moisture content in rainbow trout fillets compared to that of the control ($p < 0.001$) (Table 4). The protein content of fish in the control and experimental groups are with a similar value but is not statistically proven ($p > 0.05$). The white yarrow extract added to the feed lead to decrease in the lipid content of the rainbow trout fillet by 21.59% compared to the control group and it was statistically proven ($p < 0.01$). This result shows that the fish fed with the supplement is more useful and dietary in its quality of food for humans (Sirakov et al 2019a). The dry matter content in the meat of the experimental group of fish is lower than that of the control group by 6.07% ($p < 0.001$). The ash content of the trout fillets in the control is higher by 17.19% compared to the fish in the experimental group, but the difference was not statistically significant (Table 4).

Table 4

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

Indicator/ Group	*n*	*Moisture*	*Dry matter*	*Crude protein*	*Fat*	*Ash*
C	6	74.65±0.51	25.36±0.51	19.90±0.16	3.89±0.48	1.57±0.03
A.m	6	76.18±0.60***	23.82±0.60***	19.47±0.60	3.05±0.15 **	1.3±0.02

**P < 0.01; ***P < 0.001.

Conclusions. For the first time, the effect of white yarrow extract has been studied as a supplement to the rainbow trout diet. Fish fed with this extract have better growth indicators. The feed consumption analysis show that feed conversion ratio of the trouts from the experimental group is with 21.91% lower than that of the fish from the control group. A lower fat were measured in rainbow trout fed with *A. millefolium* supplement respectively with 21.59% compared to control fish.

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