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GROWTH PERFORMANCE AND CONDITION FACTOR OF *OREOCHROMIS NILOTICUS* SPECIES FEED WITH A DIET WHICH INCLUDE SOME PHYTO-ADDITIVES

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Abstract

The purpose of this research was to evaluate the growth performance of Oreochromis niloticus reared in a recirculating aquaculture system in case of inclusion in feed of some phytoadditives. The experiment was conducted on 98 days and the biometric measurements were performed at the beginning (I.), at the end (F.), but also at an intermediary moment (INT.-after20 days of the experiment). The experimental variants were: V1-control, V2-1% Rosmarinus officinalis, V3-1% Hippophae rhamnoides and V4-1% Zingiber officinale. At the end of the experiment the best values of IBG (1.95 g/ind.-Int.; 8.50 g/ind.-F.), SGR (1.28%/day-Int.; 0.76%/day-F.), FCR (1.37 g/g-Int.; 1.63 g/g-F.) and PER indicator (1.93 g/g-Int.; 1.61 g/g-F.) were registered in case of variant V3. Also, in same variant, were observed a reduction of the variability of body mass and total length of fish. In the variants in which phytobiotics were administered, were obtained a better condition factor (V1-2.04; V2-2.11; V3-2.07; V4-2.09) at the end of the experiment. In conclusion, the results shows that the administration of sea buckthorn in a long period of time, has the best effect on growth performance of Oreochromis niloticus species.

***Key words**: condition factor, growth performance, Nile tilapia, phytobiotics, recirculating aquaculture system.*

INTRODUCTION

In recent decades, a large part of studies has been focused on the use of medicinal herbs in the light of numerous advantages, such as sustainability and less side effects, over other immunostimulants (Mohammadi et al., 2020).
A wide range of medicinal plants show potential effects on growth and survival properties of aquatic organisms. Whole plant, parts of plant (leaf, root or seed) or extract compounds have been used as feed additives in aquaculture (Hai, 2015). The growing interest of using herbal immunostimulants in aquaculture has increased worldwide because they are easy to prepare, cheap, and they contain natural organic compounds that do not cause any threat to fish health or to human health (Talpur et al., 2013; Hai, 2015).
Generally, the primary effects of medicinal plants are to improve feed efficiency and/or daily gain (Adekunle & Oladoye, 2015), but also can be a good alternative to replace antibiotics and chemicals to prevent and control diseases (Harikrishnan et al., 2010; Vaseeharan & Thaya, 2014; Syahidah et al., 2015).
There are various reports of herbal fish diets promoting growth performance (Kim et al., 2000; Ji et al., 2007).
In aquaculture sector, the use of medicinal plants (phytochemicals) has increased significantly over the past decade for different purposes such as sex reversal compound (Gholipour et al., 2011), growth enhancer (Turan and Akyurt, 2005; Banaee, 2010; Banaee et al., 2011; Ahmadi et al., 2012; Asadi et al., 2012), immunostimulant and antipathogenic (Yılmaz et al., 2013).

Ginger (*Z. officinalis*) is generally considered as a safe herbal medicine and perennial herbaceous plant, is a part of the Zingiberaceae family (Weidner & Sigwart, 2000). They are polyphenol compounds (6-gingerol, shogaols and zingerone, alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids and minerals), which have a high antioxidant activity (Hori et al., 2003; Otunola et al., 2010; Shirin & Prakash, 2010).

Supplementing ginger in fish diets may enhance growth and will signify change in magnitude and their body composition (Talpur et al., 2013).

Sea-buckthorn (*Hippophae rhamnoides*) is a plant rich in vitamins (vitamin C, vitamin E) and carotenoids (like β-carotene and lycopene B2) and secondary plant metabolites like flavonoids (quercetin and kaempferol) which are abundant in fruit pulp and seeds (Kagliwal et al., 2012). In fish diets, sea buckthorn it is used successfully for improving disease resistance and growth performance (Todoran, 2015).

The sea buckthorn berry flour is like a fruit, its seeds and leaves are rich in nutrients and bioactive components such as vitamins, amino acids, lipids, carotenoids, xanthophyll, phenols and flavonoids and have a higher content of essential oils (Repyakh et al., 1990; Bekker & Gluschenkova, 1997; Ranjith et al., 2006; Singh et al., 2006; Yang, 2009), making it a very beneficial feed supplement for laying hens.

The sea buckthorn and its by-products are examined in recent years as a supplement in animal nutrition. Moreover, the sea buckthorn plays an important role in improving the efficiency of feed and has been used as an alternative feed, particularly in poultry, to maintain their production, performance and high-quality yield (Mohamed et al., 2018).

Sea buckthorn (*Hippophae rhamnoides*), as a functional plant homologous to medicine and food, is crammed with a variety of nutrients and biological substances, such as organic acids, polysaccharides, unsaturated fatty acids, and various amino acids required by the human body (Pichiah et al., 2012; Tamchos & Kaul, 2019). Although several studies have reported that sea buckthorn extract has the positive effect of lowering plasma cholesterol, increasing intestinal probiotics, improving lipid metabolism enzyme activity, enhancing antioxidant ability, and reducing the incidence of chronic diseases (Yuan et al., 2011; Pichiah et al., 2012; Kwon et al., 2017; Hao et al., 2019), but studies using sea buckthorn itself are limited.

Rosemary (*Rosemarinus officinalis*), belonging to the Lamiaceae family, is well known for its antioxidative properties, and is also used in several pharmaceutical applications (Cheung & Tai, 2007). Biologically, rosemary extract improved feed conversion, efficiency of broilers fed diet supplemented with such herb (Ghazalah & Ali, 2008). Cagıltay et al. (2013) reported that the parallel to the concentration of rosemary in fed has decreased crude fat and increased amount of crude protein. Also, there have been a few studies on the antioxidative effect of rosemary in fish (Perez-Mateos et al., 2002; Valeria et al., 2010; Alvarez et al., 2012). The growth performance (SGR and WG) and feed utilization (FCR and FER) of the fish increased with the increase in the rate of rosemary in the feed ($p<0.001$) (Karatas et al., 2020).

The antioxidant capacity of rosemary is attributed to three phenolic diterpenoids (carnosic acid, carnosol and rosemaric acid), but many other components (rosmanol, epirosmanol, isorosmanol, rosmaridiphenol, rosmadial, rosmariquinone, carvacrol, carvone, cymene, cineole, fenchone, limonene, terpinene and thymol) are expected to contribute to its antioxidative and antimicrobial properties (Fu et al., 2007).

The aim of this study was to evaluate the growth performance of *Oreochromis niloticus* reared in a recirculating aquaculture system in case of inclusion in feed of some phyto-additives.

MATERIALS AND METHODS

Description of the RAS Pilot Station.

The present study was conducted in RAS pilot station within "Dunărea de Jos" University of Galați, during a 98 days experimental period. The experimental intensive production system was designed and configurated according to the indications presented by Cristea (2008). The detailed designed of the aquaculture intensive production system is described in Figure 1.

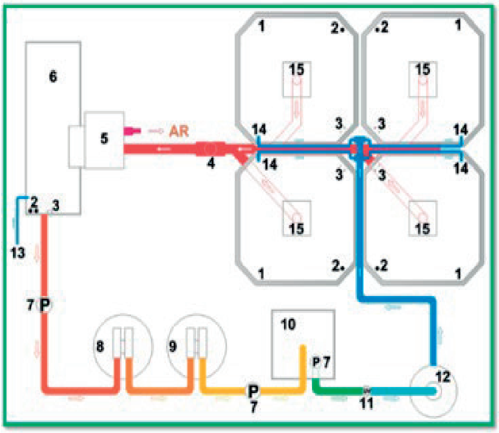

Figure 1. The design of RAS pilot station: rearing units - No. 1; nitrogen compounds sensors - No. 2; water level sensors - No. 3; RAS outlet structure - No. 4; mechanical drum filter - No. 5; sump - No. 6; pumps - No. 7; sand filter - No. 8; activated charcoal filter - No. 9; biological trickling filtration unit - No. 10; sterilization UV filter - No. 11; oxygenation unit - No. 12; automatically fresh water inlet No. 13; rearing units water inlet/outlet structure - No. 14, 15 (Petrea et al., 2020)

Design experimental

The biological material consisted in a total number of 168 individuals of *Nile tilapia*, with an initial average weight of 280.06 ± 54.02 g/fish, respectively with an initial total length of 24.25 ± 1.38 cm/fish, that were randomly distributed in four rearing units.

The experiment was conducted on 98 days and the biometric measurements were performed at the beginning (I.), at the end (F.), but also at an intermediary moment (INT. - after 20 days of the experiment).

The experimental variants were organized as follows: V1 - control, V2 - 1% rosemary (*Rosmarinus officinalis*)/kg feed, V3 - 1% sea buckthorn (*Hippophae rhamnoides*)/kg feed and V4 - 1% ginger (*Zingiber officinale*)/kg feed. These phytobiotics were purchased from a Plafar market, like dried plants, after which they were grounded and used as powder.

The additivation of fish feed with these plants was made according to the indications presented by Mogodan et al. (2020).

Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Antache et al. (2013). Fish were fed four times per day with a daily ration of 2% from fish body weight.

During the experimental period the monitored water quality parameters were kept within acceptable limits for the optimal growth of the *Oreochromis niloticus* species.

The main physico-chemical water quality parameters were monitored daily: oxygen (mg/L) with TriOxmatic 700IQ WATT (W) sensor, temperature (°C) with sensor TrioxiTherm WATT, pH with WAT Sensolyt 700 IQ (SW) sensor and the concentration of $N\text{-}NO_2$ (mg/L), $N\text{-}NO_3$ (mg/L) and $N\text{-}NH_4$ (mg/L) was also monitored twice a week using Spectroquant photometer, Nova 400.

Technological indicators

The analysed technological indicators were:

- Individual biomass gain:

$$IBG = (Bf)\text{-}(Bi)/\text{fish number [g/fish]}, (1);$$

with:
Bf - final fishbiomass,
Bi - initial fish biomass.

- Specific growth rate:

$$SGR = 100 \times (\ln Bf - \ln Bi)/t \text{ [\% fish biomass/day]}, (2);$$

with:
Bf - final fish biomass,
Bi - initial fish biomass,
t - duration of the experiment.

- Feed conversion ratio:

$$FCR = F/IBG \text{ [kg feed intake/kg fish biomass gain]}, (3);$$

with:
F - feed intake,
IBG - individual biomass gain.

- Protein efficiency ratio:

$$PER = IBG/(F*CP/100) \text{ [kg/kg]}, (4);$$

with:
IBG - individual biomass gain,
F - feed intake,
CP - crude protein.

- Condition factor:

$$K = W/L^3 \times 100, (5);$$

with:
W - body weight,
L - body length.

- Variation coefficient:

$$CV_{BW \text{ or } TL} = (\text{Dev. St.} / \text{Avg W (or L)}) \times 100 \text{ [\%]}, (6);$$

with:
Dev. St. - standard deviation,
Avg BW or TL - fish body weight or length.

Statistical methods
For the statistical analysis presented in this paper was used the software IBM SPSS Statistics 20. To determine significant differences among groups was used the one-way analysis of variance (ANOVA); $p<0.05$ was considered as significant. The homogeneity of variance was tested by using Levene's test (F value).

RESULTS AND DISCUSSIONS

When it was done the fish distribution in all four experimental variants has been taken into account the homogeneity of fish in terms of body weight (Levene test $p>0.05$).
The variability of fish body weight (g) and total length (cm) is expressed by the coefficient of variability (CV - %). The evolution of the variability coefficient of body weight (CV_{BW}) and of the total length (CV_{TL}) throughout the research period can be observed in Figure 2, respectively in Figure 3.

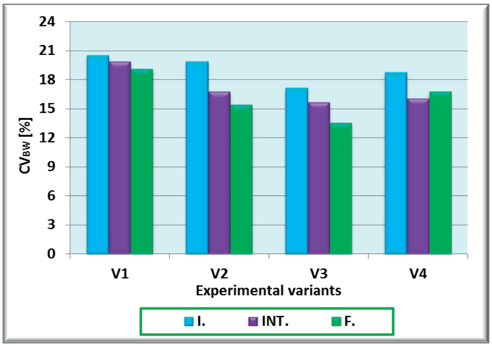

Figure 2. Evolution of variability coefficient of body weigth (CV_{BW}) throughout the experiment
Note: I. - initial, INT. - intermediary, F. - Final

During the experimental period a reduction in body weight and total length variability in all experimental variants was observed. However, at the level of body weight, this is more evident in the variants in which phyto-additives (V2, V3, V4) were administered after 98 days compared to the values recorded at the beginning of the experiment. In conclusion, this shows us a better grouping of the body weights around the average value in V2, V3 and V4 variants. In the control variant we can say that at the end of the experiment the heterogeneity of the studied group increased.

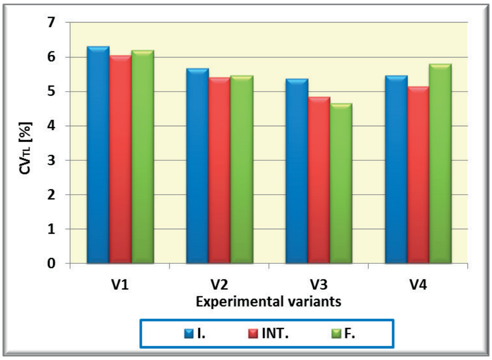

Figure 3. Evolution of variability coefficient of body weigth (CV_{TL}) throughout the experiment
Note: I. - initial, INT. - intermediary, F. - Final

The normality of fish body weight distribution values is presented using histograms. The evolution of the normality of distribution during the experiment can be observed in the Figures 4, 5 and 6.
Thus, the initial mean of fish body weight did not show significant differences by statistically point of view between the experimental variants, these values being grouped by the Duncan test in a single subset of data ($p>0.05$).

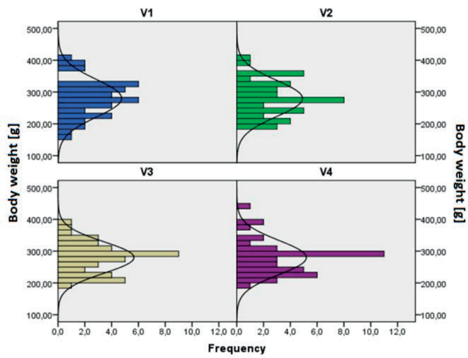

Figure 4. Histogram of the individual body weight at the beginning of the experiment (I.)

The individual mean body weight recorded at the beginning of the experiment for each experimental variant was: 280.40 ± 57.53 g/fish in V1 variant; 279.83 ± 55.58 g/fish in V2 variant; 280.05 ± 48.13 g/fish in V3 variant and 279.98 ± 52.51 g/fish in V4 variant.
Regarding to the individual average of total length between the experimental variants, no significant differences were registered ($p>0.05$), the variants being homogeneous. The

average values was 24.36 ± 1.54 cm in V1 variant, 24.26 ± 1.37 cm in V2 variant, 24.24 ± 1.30 cm in V3 variant and 24.15 ± 1.32 cm in V4 variant.

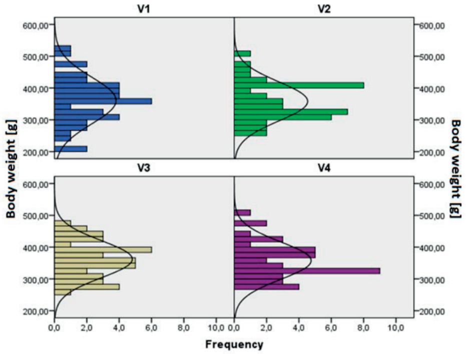

Figure 5. Histogram of the individual body weight at the intermediary moment (INT.)

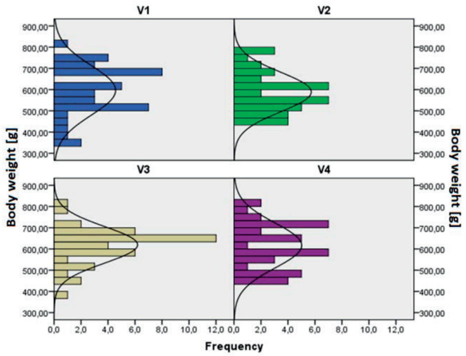

Figure 6. Histogram of the individual body weight at the end of the experiment (F.)

Following the somatic measurements performed during the experiment (INT. and F.), the boxplots corresponding to body weight and total length were also made.

The values of the median, minimum, maximum and quartile for the individual body weight and total length at the beginning of the experiment are presented in the boxplots from Figures 7, respectively 8.

Both after the intermediary measurements and at the end of the experiment a constant increase of the individual average body weight was observed, especially in the variant in which sea buckthorn (V3) was administered, but an increase was also observed in the variant in which ginger (V4) was administered, but was lower than in variant V3.

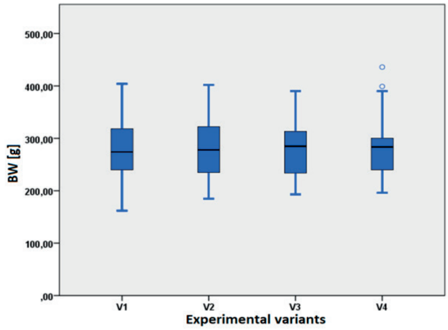

Figure 7. Boxplot of initial body weight (g)

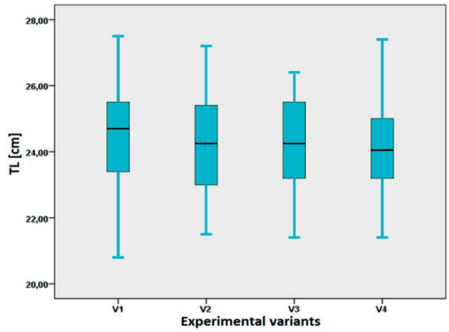

Figure 8. Boxplot of initial total length (cm)

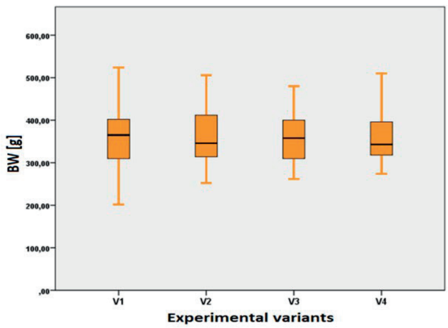

Figure 9. Boxplot of body weight (g) at intermediary moment

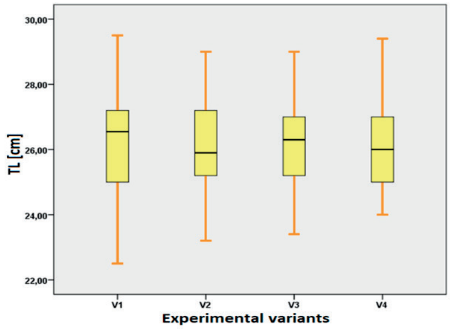

Figure 10. Boxplot of total length (cm) at intermediary moment

However, during the experimental period the increase of mean body weight in variant V3 was not significant compared to the results obtained in the other experimental variants (INT. - p>0.05; p = 0.985; F. - p>0.05; p = 0.456). Also, during the experiment, no significant differences were found in the individual total lengths of fish (p>0.05) (INT. - p = 0.875, F. - p = 0.330).
The boxplots of fish body weight for the intermediary moment and for the end of the experiment are presented in Figures 9 and 11, and for the total lengths the boxplots are showed in Figures 10 and 12.

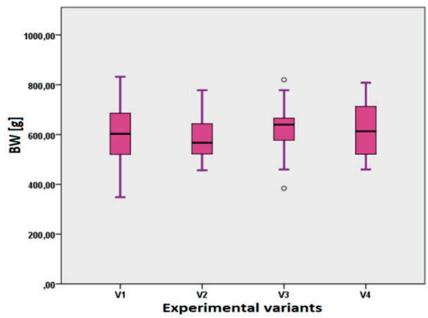

Figure 11. Boxplot of body weight (g) at the end of the experiment

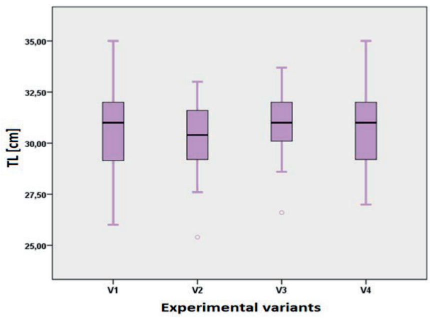

Figure 12. Boxplot of total length (cm) at the end of the experiment

The correlation between body weight and total length for the Nile tilapia exemplars was determined from each experimental variant. Therefore, the Pearson coefficient was calculated (Table 1).
Pearson coefficient indicates the degree of association between two variables, in our case it is represented by the fish total length and body weight. Thus, from the values obtained for the Pearson coefficient it can be seen that both at the beginning of the experiment and in the other experimental stages (INT. and F.) between the two variables there was a strong positive relationship, because the values of R were registered between 0.75 and 1.

Table 1. Values of Pearson coefficient during the experimental period

Experimental variants	Pearson coefficient		
	I.	INT.	F.
V1	0.904	0.854	0.864
V2	0.915	0.867	0.842
V3	0.883	0.841	0.822
V4	0.889	0.889	0.792

In the same time, the regressions were performed with which we can determine allometric factor "b" through which we can see if the fish has grown more in mass or in length. The allometric factor “b” and the determination coefficient “R^2” during the entire experiment for each experimental variant are presented in Figures 13, 14, 15 and 16.

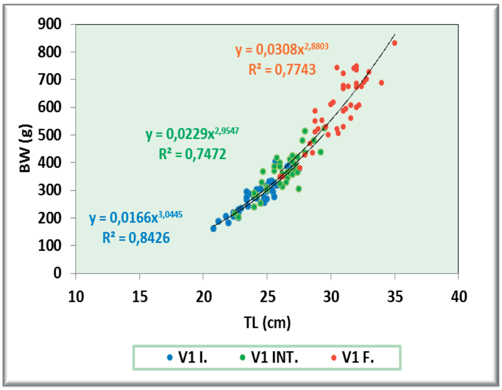

Figure 13. Power regression of individual fish length-weight from V1 variant during the experimental period

The allometric condition factor “b” shows us that during the experiment a negative allometry was registered. The values obtained show that the fish increased more in length than in body weight, because the value of "b" is less than 3. Following the analysis of the determination coefficient “R^2”, a good correlation was found between the total length and the body weight of the specimens, respectively the proportion in which the increase of the body mass can be attributed to the increase in length.

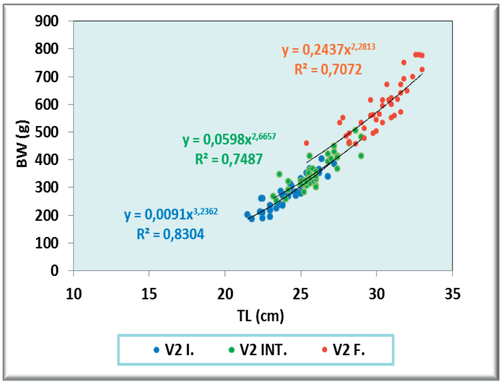

Figure 14. Power regression of individual fish length-weight from V2 variant during the experimental period

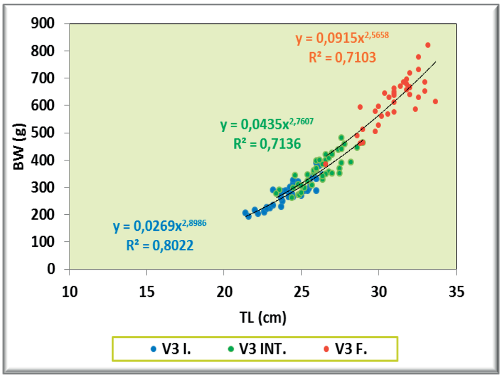

Figure 15. Power regression of individual fish length-weight from V3 variant during the experimental period

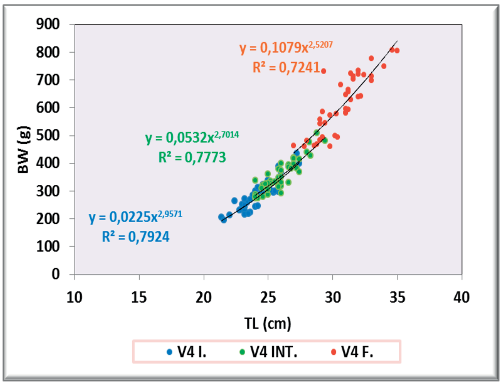

Figure 16. Power regression of individual fish length-weight from V4 variant during the experimental period

Therefore, it varied at the beginning of the experiment from a minimum of 79.2% (V4) to a maximum of 84.3% (V1). Among the variants in which phyto-additives were administered only in variants V3 and V4 was observed an increase more in body weight than in length compared with V2 variant fact also given by the value of the determination coefficient (Figures 13-16).

In aquaculture, the condition factor (Fulton`s coefficient - "K") is used in order to compare the "condition", "fatness" or well-being of fish. It is based on the hypothesis that a better physiological condition is reflected by a heavier fish of a particular length (Metaxa et al., 2018).

Table 2. Condition factor "K"at Nile tilapia during the experimental period

Experimental variants	Condition factor		
	I.	INT.	F.
V1	1.92±0.17	1.99±0.21	2.06±0.21
V2	1.94±0.17	2.02±0.18	2.11±0.21
V3	1.95±0.16	2.00±0.17	2.07±0.17
V4	1.97±0.17	2.02±0.16	2.10±0.21

Moreover, the condition factor is at the same time a measurement which shows the fact whether the fish is fed enough (Mert et al., 2008).

In variants in which the phyto-additives were administered, the highest values of the condition factor "K" were registered, which shows a good condition of the fish. But, the differences were statistically insignificant ($p>0.05$) compared to the control variant both at the intermediary moment and at the end of the experiment (Table 2).

Regarding the technological growth indicators, was registered a better growth performance in the variants in which sea buckthorn (V3) and ginger (V4) were administered compared to the other experimental variants both in the intermediary moment (INT.) and at the end of the experiment (F.) (Table 3).

It was observed that the best evolution of the stocking density (SD - kg/m^3), but also of the total biomass gain (TBG - kg/m^3) was registered in the variant in which sea buckthorn was administered (V3), followed by V4 variant in which ginger was administered. The individual average of body weight recorded the best values also in the V3 variant (INT. - 362.10 ± 56.66 g/fish; F. - 620.20 ± 84.40 g/fish) (Figures 9 and 11).

If at the intermediary moment the total biomass gain did not show visible changes with the administration of phytobiotics, at the end of the experiment the changes are much more obvious. In V3 variant was observed an increase with 2.09% at intermediary moment, respectively with 7.32% at the end of the experiment compared to the control variant (V1). Ginger administration in fish feed led to a reduction of the total biomass gain with 3.14% at intermediary moment, but and an increase with 6.31% at the end of the experiment compared to the control (V1).

Regarding to the rosemary administration, it contributed to a reduction of the total biomass gain with 2.62% in intermediary moment, respectively with 2.27% at the end of the experiment compared to the control variant (V1). Thus, the highest total biomass gain was recorded in the variant in which sea buckthorn (*Hippophae rhamnoides*) was administered, followed by ginger (*Zingiber officinale*).

The most suggestive technological indicators for growth performance show that the food conversion ratio (FCR) is indirectly proportional with the specific growth rate (SGR) and the protein efficiency ratio (PER). Their best values were registered in the variant in which sea buckthorn was administered in fish feed, being followed by those obtained in the variant with ginger administration and in the control variant. The administration of rosemary did not lead to an improvement in growth performance, fact evidenced by the results obtained from the SGR, FCR and PER determination.

Table 3. Growth performance indicators for each of the experimental variants

Technological indicator	Experimental period	Experimental variants			
		V1	V2	V3	V4
Fish stocking density (kg/m^3)	I.	29.44	29.38	29.41	29.4
	INT.	37.87	37.59	38.02	37.54
	F.	59.72	58.93	62.02	61.69
Total biomass gain (kg/m^3)	I.- INT.	8.43	8.21	8.61	8.14
	INT. - F.	21.85	21.34	24	24.15
	I. - F.	30.28	29.55	32.61	32.29
Individual biomass gain – IBG (g/fish)	I. - INT.	1.91	1.86	1.95	1.85
	INT. - F.	5.66	5.64	6.02	5.95
	I.-F.	7.92	7.74	8.5	8.42
Specific growth rate - SGR (%/day)	I.- INT.	1.26	1.23	1.28	1.22
	INT. - F.	0.58	0.58	0.63	0.64
	I. - F.	0.72	0.71	0.76	0.75
Feed conversion ratio - FCR (g/g)	I.- INT.	1.4	1.43	1.37	1.44
	INT. - F.	1.87	1.88	1.73	1.7
	I. - F.	1.74	1.76	1.63	1.64
Protein efficiency ratio - PER (g/g)	I.- INT.	1.88	1.84	1.93	1.82
	INT. - F.	1.41	1.4	1.52	1.54
	I. - F.	1.52	1.5	1.61	1.61

Note: I. - beginning of the experiment, INT. - intermediary moment, F. - final of experimental period.

Regarding to the effect of sea buckthorn on technological indicators of growth performance Csep and Bud (2010) reported similar results at *Cyprinus carpio* species. The research carried out by them consisted in the existence of three experimental variants: L1 - control variant, L2 - variant in which the diet was supplemented with 1% sea buckthorn/kg feed and L3 - variant in which the carp diet was supplemented with 2% sea buckthorn/kg feed. The results obtained by these showed that the administration of sea buckthorn in a concentration of 1%/kg feed led to the improvement of growth performance to a greater extent than in the variant L3, respectively L1 (Csep & Bud, 2010).

The ginger administration in 1% concentration per kg of feed contributed to a better optimization of growth than that obtained in the control variant and in the variant in which rosemary was administered, but lower than that obtained in variant in which sea buchthorn was administered. Better results of the feed conversion ratio and an increase of body weight, compared to the control variant, were also registered in the case of the diet supplemented with ginger in case of rainbow trout (Nya & Austin, 2009; Gabor et al., 2011). Another study provides evidence that dietary administration of ginger in 0.8% concentration for 60 days can modulate growth performance at *Labeo rohita* species (Sukumaran et al., 2016).

The growth enhancement following ginger administration in fish feed can be attributed to stimulated secretion of intestinal proteases by the host, thus improving digestion and absorption of proteinous components of the feed (Mohammadi et al., 2020). Besides, ginger rhizomes as a rich source of proteinase, enhance proteins digestion and amino acid absorption in the gastrointestinal tract (Hashim et al., 2011). Ginger also has positive effect on intestine probiotic bacterial flora and aid in gaining even more nutrients (Ali et al., 2008).

Concerning to the supplementation of the diet with 1% rosemary/kg feed, a reduction of the technological indicators of growth performance in Nile tilapia was observed compared to the results obtained in the control variant at the end of each experimental period (INT. and F.). At the *Dicentrarchus labrax* species, the same aspect of decreasing the values of growth indicators was observed in the case of rosemary administration in 1% concentration in feed compared to the control variant (Yilmaz et al., 2012). Instead, Yilmaz et al. (2011) showed that the addition of rosemary extracts promoted growth and enhanced some nonspecific immunity indicators at tilapia, *Oreochromis mossambicus* and study of Turan & Yigitarslan (2016), established the efficacyty of rosemary extract feed additives as a growth promoter in *C. gariepinus*.

CONCLUSIONS

The determination of growth performance indicators, such as the specific growth rate, the feed conversion ratio, the protein efficiency ratio and the biomass gain, indicated that the best values were recorded in the variant in which sea buckthorn was administered, followed by the variant in which ginger was administered. But, the effect of ginger on growth was superior to the effect of rosemary.

Although, the experiment was carried out over a longer period of time (98 days), the research showed that the administration of rosemary did not contribute to the improvement of growth performance at Nile tilapia in our case.

The weight-length relationship, fish distribution and the condition factor values, presented in this research, provides useful information in terms of fish production technology and fish population dynamics during a growth production cycle. Following the analysis of the variability coefficient, a reduction of body weight variability was found in variants V2, V3 and V4, especially at the end of the experiment, respectively after 98 days of experiment. Also, a better condition factor was registered in variants in which were administered phyto-additives (V2, V3 and V4).

In conclusion, the results shows that the administration of sea buckthorn in a long period of time, has the best effect on growth performance of *Oreochromis niloticus* species.

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