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Disease resistance effect of sea-buckthorn (*Hippophae rhamnoides* L.) added in the fish diet

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Abstract. In this paper, we approached a research theme regarding the effects of sea-buckthorn *Hippophae rhamnoides* L. added in the diet the common carp *Cyprinus carpio* upon the disease resistance in case of the imminent disease outbrake due to water condition changes. This research theme was approched after studying the references according to which in many cases the sea-buckthorn added in the diet of the farmed animals, led to good health conditions and resistance against some pathogen agents. Unfortunately, the studied references do not mention the same facts in case of aquatic organisms. In order to make possible our study, at the end of an experiment which followed the effects of sea-buckthorn upon the growth and development of the common carp in pond conditions, we took from each experimental lot 10 individuals and transferred them in laboratory conditions. The fishes were kept in aquariums each with a total capacity of 500 L. Feeding was made whith the same feeds experimented in the pond based experiment, as follows: Lot I - control feed without sea-buckthorn adding; Lot II - feed whith 1% sea-buckthorn, and Lot III was fed with feed containing 2% sea-buckthorn. The experiment took place in a relatively short period of time, between 18.10.2010-12.11.2010. In this period of time, we followed the effect of sea-buckthorh added to the fish meal, upon the resistence against some pathogen agents which infect fishes once the water parameters are changed. The results obtained, at the end of our study, makes us support the use of sea-buckthorn in the diet of fishes in order to stimulate the resistance against some diseases.
Key Words: sea-buckthorn, carp, water condition changes, disease, immunostimulant, health.

Összefoglalás. Jellenlevő dolgozatunk végkitűzése az volt, hogy kimutajuk a haltápba adalékolt homoktövis hatását, a ponty imunrendszerének gyarapítására és egyben az életkörülmények változásával járuló betegségekkel szembeni ellenállását. Kutatási témánk választásához hozzájárult a szakirodalom tanulmányozása, mely szerint a homotövis használata a háziállatok takarmányozásában pozitív irányba fordítja ezek egészségügyi állapotát. Sajnos a szakirodalom kutatása során, nem találtunk adatokat melyek a homoktövis, haltépban való használatáról szolnának, tény mely alátámasztotta kutatási témánk helytállósát. Kutatásunk végrehajtása végett, egy eloző kisérletünk lezártakor-mely során a homoktövis hátását vizsgáltuk togazdasági körülmenyék közt- mindegyik kisérleti csoportból, laboratoriumba szálítottunk 10-10 egyent. A halak ujracsoportosítása 500 l-es akváriumokba történt, fojtatva a tógazdaságban történő takarmányozást: I csoport, alaptad homoktövis adalék nélkül; II.csoport, alaptáp+ 1% homoktövis; III. csoport alaptáp+ 2% homoktövis. Kisérletünk 26 nap folyamán lett kivitelezve, 2010.10.18-2010.11.12 közt, időtartam mely során vizsgálataink a homoktövis hatását célozták a halak betegségek iránti ellenállás gyarapitására. Kutatásaink során elért eredmélyeink arra biztatnak, hogy a homoktövis használatát a halak táplálékában támogatásba reszesítsük, léven hogy e adagolás során sikeres módon gyarapíthattuk a halak védekezesi képessége bizonyos betegségek ellen.
Kulcsszavak: homoktövis, ponty, életkorülmélyek változása, betegség, immunrendszer-gyarapítás, egészség.

Rezumat. În lucrarea de faţă ne-am propus evidenţierea efectelor adaosului de cătină din hrana crapului, asupra stimulării rezistenţei faţă de unele boli iminente odată cu schimbarea mediului de viaţă. Această tematică de cercetare a fost aleasă în urma studierii literaturii de specialitate, care tratează în multe cazuri efectele benefice ale cătinei asupra stării de sănătate a organismelor terestre, referinţele pentru organismele acvatice fiind aproape inexistente. Pentru realizarea prezentul studiu, la finele unui experiment desfăşurat în condiţii de heleşteu, care a vizat efectele cătinei asupra performanţelor de producţie la crap, s-au transvazat în condiţii de laborator câte 10 indivizi din fiecare lot experimental. Relotizarea materialului biologic a avut loc în acvarii a câte 500 L fiecare, hrănirea loturilor astfel create, s-a efectuat identic cu hrănirea de pe parcursul experimentului din teren, lotul I beneficiind de furaj fără adaos de cătină, lotul II de un adaos de 1%, respectiv lotul III a fost hrănit cu acelaşi furaj de bază, însă cu un adaos de 2% cătină. Experimentul s-a desfăşurat pe o perioadă relativ scurtă, de 26 zile, între 18.10.2010 și 12.11.2010, interval în care s-a urmărit efectul cătinei din furajul administrat asupra rezistenţei la anumiţi agenţi patogeni care pot apărea odată cu schimbarea parametrilor fizico-chimici ai apei. Rezultatele obţinute în urma acestui experiment ne permit recomandarea utilizării cătinei în raţia furajeră a peştilor în general şi al crapului în special, adaosul de cătină din hrana administrată ajutând la stimularea sistemului imunitar.

Cuvinte cheie: cătină, crap, schimbare de mediu, boală, imunostimulator, sănătate.

Introduction. The immune system plays a decisive role in both the etiology and the pathophysiological mechanism of various diseases. Activity (its effectiveness) is closely interrelated with many exo and endogenous factors (food, pharmacological products, stress) that have the purpose or effect of immunostimulant or immunosuppressant (Dale & Foreman 1989).

Far from having a specific effect of stimulating or suppressing, some agents, used in pharmacological activity, have been shown to influence pathophysiological processes which is why they were called immunomodulatory agents (Patwardhan et al 1991). Often these immunomodulatory natural agents, being represented mainly by herbs, which from immemorial time have been used in traditional medicine, will find their place in modern medicine.

The use of plants in traditional medicine has proved that they have a strong potential to influence the immune response (Li et al 2003). In this plant category falls the sea-buckthorn (*Hippophae rhamnoides* L.), whose immunomodulatory properties have been known since antiquity and it is commonly used in treating certain health conditions or merely prophylactic agent. In this context we considered appropriate to address the topic to track the impact on health and immunomodulation in fish.

Sea buckthorn is a thorny shrub, native to Europe and Asia which until recently has been used in traditional medicine, but just in the last decades came to the attention of scientists. During time the sea-buckthorn has been used extensively against cardiovascular disease, gastrointestinal dysfunctions treatment, treating injuries, skin burns, and other diseases more or less critical (Brad 2002).

Composition of sea buckthorn range of bioactive substances has led the development of interest on this plant, finding always new and interesting effects (Brad 2002). It should be noted that all parts of sea-buckthorn are a rich source of bioactive components, their highest concentration being found in fruit (vitamins A, C, E, K, carotenoids, organic acids etc, Yang & Kallio 2001).

Sea-buckthorn was used as an immunomodulator agent in case of animal breeding and veterinary medicine, the outcomes being spectacular, fact that led to the administration of sea buckthorn in animal feeds in order to prevent some health problems (Morar 2003). To support these claims, we can mention the work "Sea buckthorn, a pharmacy in a plant", Brad (2002), the author delivers spectacular results in treating the stress accommodation disorders in case of some imported animals, and treatment of necrobacillosis and skin diseases in sheeps, and cows mastitis.

Like farmed animals, fish are also vulnerable to some pathogen agents, the treatment of the disease caused by them, being hard, in most cases impossible (Negrea 2007).

The outbreak of illness in fish, is not only the result of the action of the pathogen agent. For the disease to manifest itself, the fish organism must be receptive, the state of receptiveness and the organism resistance being both strongly influenced by environmental conditions (Bud et al 2010).

Regarding the aquatic environment, it can influence the appearance of some diseases by its stressful activity upon the fish organism, stressful state being the condition in wich the fish is under disconfort, under the action of adaptive mechanisms (Marcu et al 2010).

Stressors in case of aquatic vertebrates are very numerous, which by their singular or accumulated action determines the state of disease. Among the many stressful factors can be mentioned: temperature fluctuations, fishing, water chemistry, organic loading of the aquatic environment, transport, handling, re-houseing the animals in new pond or other farming systems etc (Sas & Covaciu-Marcov 2007; Sas et al 2009; Petrescu-Mag & Petrescu-Mag 2010; Taati et al 2010).

In such circumstances, hormonal balance is disturbed, reducing the resistance of fish, facilitating the triggering of parasitic diseases, fungal, infectious or others. Often, in these conditions of stress, even a weaker pathogen which invades the host causes serious illness (Munteanu & Bogatu 2003; Petrescu-Mag et al 2007).

Our research analyzed the two summer old carp resistance to some pathological agents, wich appear inevitably following the state of stress determined by the changing of the living enviroment, when sea-buckthorn was added in its diet.

Material and Method. From April 1 to October 15, 2010, in the farm Piscicola LLC Cehu-Silvaniei, an experiment was conducted that focused on the effects of sea-buckthorn upon the growth performances of the common carp in pond conditions.

The three lots from that experiment, were bread under the same medial conditions, the only variable between the groups being represented by the feed used (see Table 1).

Table 1

Basic and experimental diet composition

Element	M.U.	Tested fish diets		
		L1-basic	*L2*	*L3*
Corn	%	30	29	28
Wheat	%	20	20	20
Soybean meal	%	27	27	27
Sunflower meal	%	15	15	15
Fish meal	%	5	5	5
Dicalcium phosphate	%	2	2	2
Premix	%	1	1	1
Sea-buckthorn	%	-	**1**	**2**
TOTAL	%	100	100	100

At the end of the experimental period, a part of the biological material under study was carried in the aquaculture laboratory of USAMV Cluj, and the individuals subjected to this study were re-grouped. To achieve this experiment, we used three aqariums with a total capacity of 500 L each. In these tanks, there were organised the experimental lots, each lot being composed of 10 individuals, with an average weight of 310.61g (Figure 1). Feeding was made with the same granulated feed, wich was used in the pond experiment (Table 2).

Table 2

Chemical composition of the tesed feeds

Element	M.U	Measured values			
		Sea-Buckthorn	*L1 control*	*L2 1% SBT*	*L3 2% SBT*
Crude protein	%	10.3	22.6	23.37	24.8
Crude fat	%	16.83	2.76	3.15	3.40
Crude fiber	%	9.25	4.51	4.72	4.97
Crude ash	%	2.20	7.54	5.95	6.18
Ca	%	0.09	1.02	0.80	0.84
P	%	0.10	1.28	1.00	1.15
Mg	%	0.07	0.26	0.25	0.25
Na	%	0.04	0.11	0.06	0.06
K	%	1.09	1.09	1.11	1.14
Vit. A	UI/kg	7771	4920	7700	7870
Vit. E	mg/kg	216	59	65	71
Total carotenoids	mg/100g	54	0.38	0.47	0.48

Note: SBT - sea-buckthorn

Figure 1. Moving the biological material from the pond conditions into the experimental conditions

The experiment was conducted over a relatively short period of 26 days, between 10/18/2010 to 11/12/2010. In this time, we studied the effect of feed administered sea-buckthorn on resistance to certain pathogens that may arise with the changing physico-chemical parameters of water. Of these parameters the temperature plays a crucial role, which adjusts the intensity of physiological processes with direct effects on health, defending ability of fish that are already weakened due to stress adaptation.

For this reason, we rised the water temperature to 25°C, in this way, the temperature being higher with 6-7°C than the water of the pond. Through this water temperature manipulation was stimulated the activity of the pathogen agents, wich did not make their appearence in the pond condition.

As was mentioned earlier, the purpose of this study was to determine whether the sea-buckthor added in the fish diet has an immunomodulatory effects. This goal was achieved by monitoring the occurrence of various diseases and their evolution, the number of mortality caused by them, the survival rate at the end of the experimental period.

Results and Discussion. After 24 hours from the re-groupment of the biological material, the fish started to show interest in the feed given, the state of stress due to accommodation to the new environment began to lose from its intensity. This statement is supported by the congestion of the fishes to the point of feeding, when the time of feeding approached and the keeper was close to the aquarium.

In case of the control lot (Lot I), which had no sea-buckthorn in its diet, the active feeding lasted 10 days (19-28.10.2010), after that the interest in the given feed lost from its intensity, and just after two days (29.10.2010) appeared the first signs regarding the infestation with saprolegniosis. As clinical signs of the disease, there were observed the whitish hyphaes on the surface of the body and fins, apathic swiming, and the fishes were gathering to the source of oxigen. The diagnosis was made clinicaly, and the microscopical analysis confirm the disease (see Figure 2).

Analyzing the data presented in Table 3, we see that in case of the control lot (Lot I), the first death occurred after 15 days (11/02/2010) from the re-groupment of the fishes, and after other two days (04.11.2010) we recorded mass mortality. Based on clinical analysis it has been determined that this mortality was due solely to saprolegniosis.

Turning our attention to the evolution of body weight, we can say that due to saprolegniosis, from the time of the feed refusal to the time of death, the biological material lost an average weight of 3.45 g.

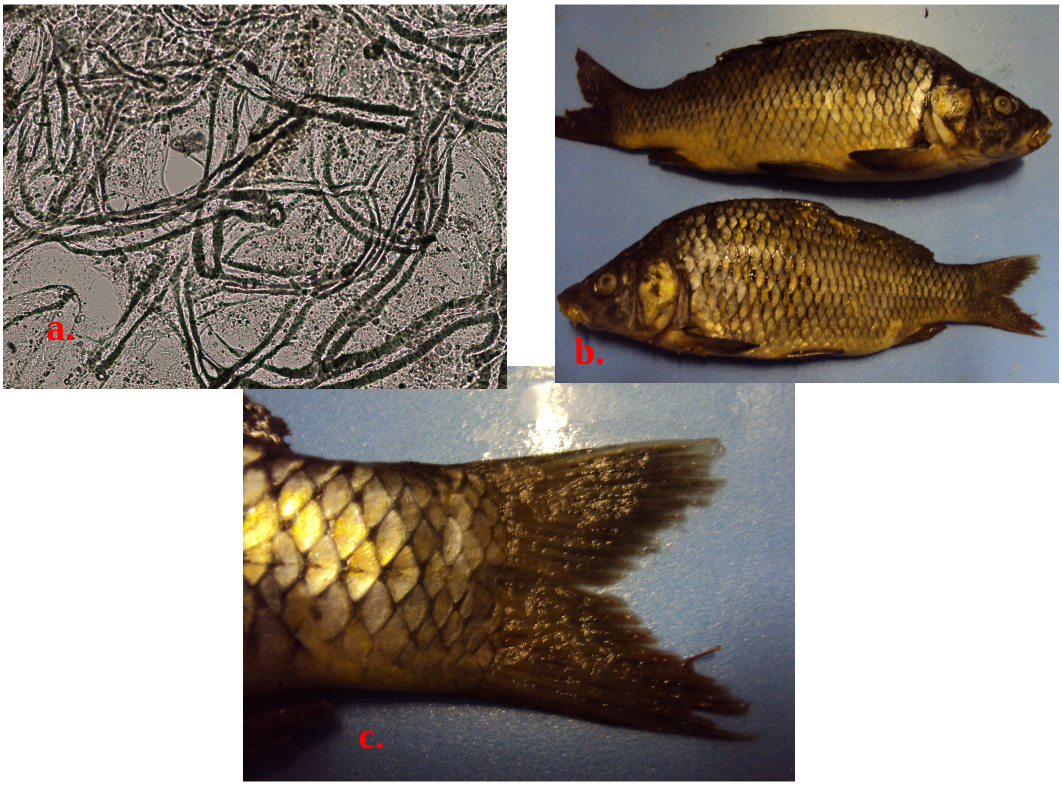

Figure 2. Clinical exam: a - *Saprolegnia* spp evidenced by microscopic analysis of the tegumentary scraping (20x). b - clinical apperance of the deceased fish, due to *Saprolegnia* spp. C - *Saprolegnia* spp. hyphae on the caudal fin

Table 3

Evolution of resistance against some pathogen agents of the individuals from Lot I (control)

Individual number	Initial weight - 18.10.2010- (g)	Cause of death			Day/date of death	Weight after death (g)	Final weight of live individuals -13.11.2010- (g)	BWA
		BHS	*S*	*NPC*				
1	251.49		x		17/ 04.11.2010	248.47	-	-3.02
2	261.22		x		15 / 02.11.2010	258.08	-	-3.13
3	285.70		x		17/ 04.11.2010	282.27	-	-3.43
4	206.75		x			204.26	-	-2.48
5	288.34		x			284.87	-	-3.46
6	279.95		x			276.59	-	-3.36
7	349.16		x			344.97	-	-4.19
8	461.56		x			456.02	-	-5.54
9	269.32		x			266.08	-	-3.23
10	223.10		x			220.42	-	-2.68
Mean	***287.659***					***284.20***		***-3.45***

Note: BHS - bacterian hemorrhagic septicemia; S - saprolegniosis; NPC - non-pathogen causes; BWA - body weight accumulation

Regarding the situation of the other experimental groups, at the time of total mortality of the control lot, we did not observed any health disorders, feeding continued normaly the biological material showing good overall condition.

At the periodic analysis of the fishes from the lot II, wich had 1% sea-buckthorn in their diet, on the 14-th day from the re-groupment, there were detected two individuals with skin ulcers. This hemorrhagic lesions were sampled and analyzed in the microbiology laboratory of the Faculty of Veterinary Medicine - USAVM Cluj, the results showed the presence of *Aeromonas hydrophila* and *Pseudomonas aerugirosa* which causes the bacterian hemorrhagic septicemia (BHS) (see Figs 3-4).

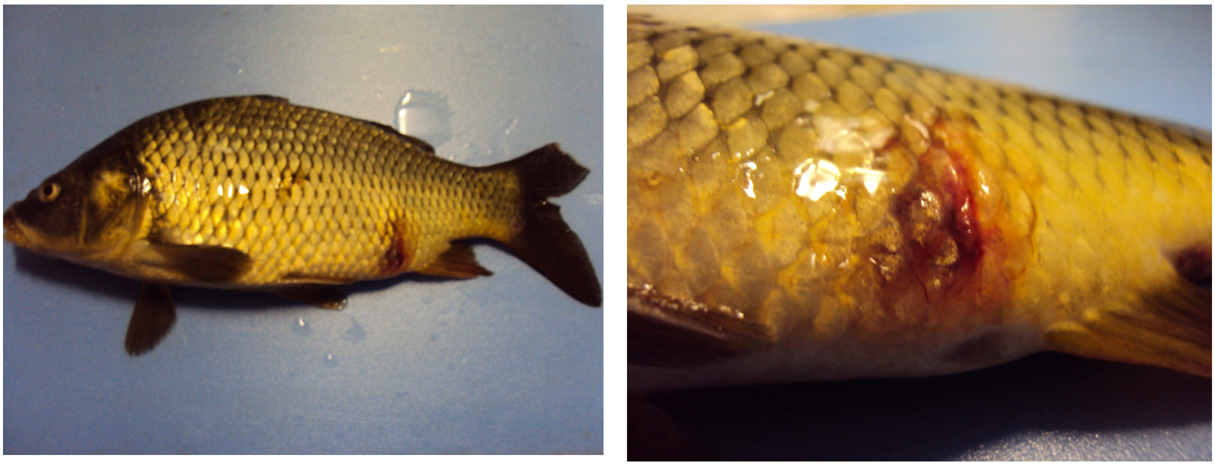

Figure 3. Tegumentary ulcers due to *Aeromonas hydrophila*

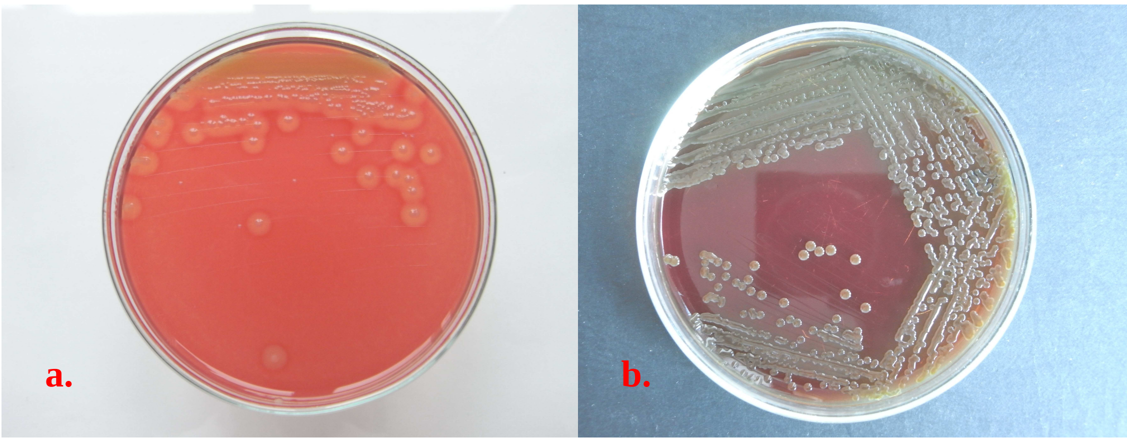

Figure 4. a- *Aeromonas hydrophila* - culture characteristics on agar with blood
b- Pseudomonas aerugirosa- culture caracteristics on agar with blood

The two individuals diagnosed with BHS, were found dead on 11/11/2010, 10 days after diagnosis (see Table 4).

Table 4

Evolution of the resistance against some pathogen agents of the individuals from Lot II

Individual number	Initial weight - 18.10.2010- (g)	Cause of death			Day/date of death	Weight after death (g)	Final weight of live individuals -13.11.2010- (g)	BWA
		BHS	*S*	*NPC*				
1	313.14	x			24/ 11.11.2010	315.02	-	+ 1.88
2	314.48						318.57	+4.09
3	373.64						378.50	+4.86
4	345.01						351.22	+6.21
5	348.75						354.85	+6.10
6	270.90	x			24/11.11.2010	272.39	-	+1.49
7	352.08						357.36	+5.28
8	418.34			x	22/ 09.11.2010	422.31	-	+3.97
9	284.82						290.23	+5.41
10	417.86						424.71	+6.85
Mean	***343.90***						***353.63***	***+5.54*/*** +2.44**

Note: BHS - bacterian hemorrhagic septicemia; S - saprolegniosis; NPC - non-pathogenic causes; BWA - body weight accumulation. *****- Mean of the live individuals body weight; **- Mean of the dead individuals body weight

If we refer to the evolution of body mass during the experimental period, in case of Lot II, we can emphasize that its evolution had an ascending trend, at the end of the study period the fishes recording an average increase of 5.54 g.

Finally, analyzing the situation of the third lot, which received an addition of 2% sea-buckthorn in its diet, we conclude that in this case, losses occurred only due to non pathologic causes (jump out from the aquarium). On the other hand, during experimental period in Lot III, were not reported any bacterial or fungal disease (see Table 5)

Table 5

Evolution of resistance against some pathogen agents of the individuals from Lot III

Individual number	Initial weight -18.10.2010- (g)	Cause of death			Day/date of death	Weight after death (g)	Final weight of live individuals -13.11.2010- (g)	BWA
		BHS	*S*	*NPC*				
1	277.55						282.82	+5.27
2	336.24						340.44	+4.20
3	292.03			x	25/ 12.11.2010	295.88		+3.85
4	301.10						306.82	+5.72
5	292.90						298.70	+5.80
6	312.10						316.63	+4.53
7	274.88						279.50	+4.62
8	321.74						326.47	+4.73
9	324.13			x	25/ 12.11.2010	327.99		+3.86
10	270.28						275.39	+5.11
Mean	***300.29***						***305.06***	+4.99*/ +3.85**

Note: BHS- bacterian hemorrhagic septicemia; S- saprolegniosis; NPC- non-pathogenic causes; BWA- body weight accumulation. *****- Mean of the live individuals body weight; **- Mean of the dead individuals body weight

Summarizing the development and etiology of mortality depending on the used feeds, we can conclude that the addition of sea-buckthorn in the fish diet, has contributed significantly to creating a higher immunity and preventing some potential disease (see Table 6).

Table 6

Synoptic table regarding the mortalities from the three experimental lots

Element	Number of individuals	Number of dead individuals	Cause of death			Death rate (%)		
			BHS	*S*	*NPC*	*Pathologic*	*Non-pathologic*	*Total*
Lot I-control	10	10	0	10	0	100 %	-	100%
Lot II- 1% SBT	10	3	2	0	1	20%	10%	30%
LotIII- 2% SBT	10	2	0	0	2	-	20%	20%

Note: SBT - sea-buckthorn; BHS - bacterian hemorrhagic septicemia; S - saprolegniosis; NPC - non-pathogenic causes; BWA - body weight accumulation

Conclusions. Through this simple experiment, but also of great importance to production, we were able to demonstrate the appropriateness of the use of sea-buckthorn in the diet of the common carp, the fruit being a promoter of resistance against some diseases.

When referring to saprolegniosis, a disease quite common in cyprinid farms, we can say that the two tested proportions of sea-buckthorn added in the fish diet head a positive effect on the resistance to this disease.

If we refer to BHS, the emmergence of this disease had a low intensity, which leads us to suggest that the addition of 1% sea-buckthorn in the fish diet, results increased resistance to the action of *Aeromonas hydrophila*, but not enough to prevent the clinical expression of this disease.

Compared to the two groups already mentioned, the third group, which received an addition of 2% sea-buckthorn in the feed, has proven the highest resistance, with no obvious disease signs, and whithout dead individuals in the lot, confirming the working hypothesis being addressed.

As a final conclusion, the results of this study allows the conclusion that the addition of sea-buckthorn in the carp nutrition improves the overall health, acting as an immunostimulant, preventing diseases occurring with the environmental changes. Based on the results of this study, we recommend the use of sea-buckthorn in the fish feeds, which will ensure a good resistance against the inevitable diseases which come once the water parameters changes.

References

Brad I., Brad I. L., Radu F., 2002 [Sea-Buckthorn, a Pharmacy in a Plant]. Editura Tehnică, Bucharest. [In Romanian]

Bud I., Vlădău V., Nădăşanu M., 2010 [Treaty for Keeping Fish]. Editura Texte, Dej, Romania. [In Romanian]

Dale M. M., Foreman J. C., 1989 Fan TD: Textbook of Immunopharmacology, 3rd Edition. Blackwell Scientific Publication, London.

Li T. S. C., Beveridge T. H. J., 2003 Sea Buckthorn (*Hippophae rhamnoides L.*): Production and Utilization, NRC Research Press, Ottawa, Ontario, 133p.

Marcu A., Marcu A., Nichita I., 2010 [Compendium of Ichtyopathology, Part I. Viral and Bacterial Diseases]. Editura Brumar, Timişoara. [In Romanian]

Morar R., 2003 [Eridiarom, Erisol, Polivitarom or Herbal Alternatives]. Editura Todesco, Cluj-Napoca. [In Romanian]

Munteanu G., Bogatu D., 2003 [Treaty of Ichtyopathology]. Editura Excelsior Art, Timişoara. [In Romanian]

Negrea O., 2007 [Fish diseases]. Editura AcademicPres, Cluj-Napoca. [In Romanian]

Patwardhan B., Kalbag D., Patki P. S., Nagsampagi B. A., 1991 Search of immunomodulatory agents - a review. Indian Drugs **28**:249-254.

Petrescu-Mag I. V., Petrescu-Mag R. M., 2010 Heavy metal and thermal stress in fishes: The implications of HSP in adapting and acclimation to different environments. Metalurgia International **15**(10):107-117.

Petrescu-Mag I. V., Botha M., Petrescu-Mag R. M., 2007 Heat shock proteins in fish - a review. ELBA Bioflux, Pilot (**a**):1-10.

Sas I., Covaciu-Marcov S.-D., 2007 Overwintering whithout winter: the exceptional case of two terrestrial anurans from the thermal habitats in north-western Romania. North-West J Zool **3**(2):121-126.

Sas I., Kovács E.-H., Covaciu-Marcov S.-D., 2009 Are the hibernating water frogs from *Pelophylax (Rana) esculentus* complex (from North-Western Romania) able to adapt to the thermal water conditions? AES Bioflux **1**(1):37-41.

Taati M. M., Jafaryan H., Mehrad B., Golpour A., 2010 Changes of ovarian fluid compositions and sperm quality parameters in koi (ornamental *Cyprinus carpio*) during spawning season. ABAH Bioflux **2**(2):83-90.

Yang B., Kallio H. P., 2001 Fatty acid composition of lipids in seabuckthorn (*Hippophae rhamnoides L.*) berries of different origins. J Agric Food Chem **49**:1939-1947.

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