EFFECTS OF VITASIL ON PROXIMATE COMPOSITION AND SOME BIOCHEMICAL PARAMETERS OF COMMON CARP (*Cyprinus carpio L.*)

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ABSTRACT

This study was conducted to evaluate the effects of VitaSil medicine on meat and some biochemical parameters of common carp (Cyprinus carpio L.). Two hundred and forty carp were randomly allocated into three groups A, B and C. Fish were induced obesity by feeding with high fat diet for 9 months. Simultaneously fish were treated with VitaSil medicine in dose 50g/kg. Blood samples were taken at the 3rd month and 9th month in order to evaluate cholesterol and triglyceride concentrations. At the end of study six fish from each of the groups were slaughtered and proximate composition of meat were determined. The results suggest thatVitaSil administration able to decrease the degree of obesity and fatty liver diseases.

INTRODUCTION

Nutrition is one of the most important factors in growth, development and maintenance of fish health. Nowadays, there is trend toward the development of using alternative ingredients particularly those from plants. The positive effect of medicinal plants used as feed additive can be expressed through to the better appetite, improve feed conversion, growth performances and meat quality (Liem, 2004).

The name for an extract of Milk thistle (*Silybum marianum*) is silymarin, who is a naturally occurring polyphenolic flavonoid and in recent years used in the aquaculture practice. Several constituents of silyamarin such as silybin (also called silybinin), isosilybin, dihydrosibilyn, silydianin, and silychristin (Luper, 1998) have been reported to have antioxidative (Banaee et al., 2011; Dan et al., 2012), immunostimulatory (Alishahi et al., 2011; Ahmadi et al., 2012; Koynarski et al., 2014), radio protective effect (Adhikari et al., 2010; 2012; 2013) and protect liver cells from chemical toxins (Arora et al., 2014; Banaee et al., 2015). Silymarin has been shown to have the effect of growth parameters of fish (Atanasov et al., 2011; Dan et al., 2012; Atanasoff et al., 2012).

The aim of the study was to evaluate the effect of different dosses of vitasil in diet of common carp on proximate composition and some biochemical parameters in the blood pertaining to carp meat quality.

MATERIAL AND METHOD

The experiments were performed under approval of the Animal Ethics Committee at the Faculty of Veterinary Medicine, Trakia University, Bulgaria by permit №21/20.01.2009.

Fish were randomly divided into three groups (n = 240) and fed either a control diet (Soprfish 32/8 Profi, Veterinarski zavod, Subotica, Serbia) and a high protein and fat diet (Soprofish 48/12 HP, Veterinarski zavod, Subotica, Serbia) with or without VitaSil medicine for nine months. The Group A were fed only a high protein and fat diet. The Group B were fed a high protein and fat diet and supplemented with 50 $g.kg^{-1}$ VitaSil. The VitaSil is patent medicine containing silybin and fat-soluble vitamin E (50 mg) and vitamin A (1500 UI). The Group C (control group) were fed a control diet.

The fish were electro-anesthetized by method described Çağıltay et al., (2015). Out of water, fish handling and blood samples were took by wearing latex gloves to minimize damage to the skin and mucus covering. Fine mesh nets were used for catching and after induction the carps were placed on a non-abrasive surface, such as waterproof drape to prevent damage to delicate piscine cuticle. Blood was drawn from the *vena caudalis* using a needle (18G) in container with heparin as anticoagulant. The blood was collected (0,5-1,5 ml) in Eppendorff vials and centrifuged (3000 rpm for 15 min, Janetzki T30 centrifuge). The triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined with commercial kits (Giesse, Diagnostics, Italy) on an Automatic Biochemistry Analyzer (BS-3000P, Sinnowa Ltd., Nanjing, China).

The end of study, 6 carp from different group were sacrificed and meat were collected for proximate analysis. The samples were prepared AOAC (2006; method 983.18) and subjected to moisture analyses using air drying AOAC (1997; method 950.46). Crude protein content was calculated by converting the nitrogen content by multiplying by 6,25 due to the fact protein is 16 percent nitrogen (100/16 = 6,25), determined by Kjeldahl's method using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content was determined by the method of the Soxhlet using an automatic system (Soxtec 2050, FOSS, Sweden).

Statistical analyses were performed using SPSS statistical software (version 19.0; SPSS Inc., Chicago, USA). All significance tests were two-tailed and P<0,05 was considered significant.

RESULTS AND DISCUSSION

Meat quality is significantly influenced not only by the species, the sex, the live weight, the stress factors at slaughtering and the technological processing of the corpse, but also by the diet. The impact of the feed on the quality of the meat is determined primarily by the content of fat and carbohydrates in them (D'Souza and Mullan, 2002).

Table 1

Proximate composition of the meat from different groups

Component	Group	3rd month	9th month	Reference[1]
Moisture (g.kg^{-1})	Group C	742,2±0,56	768,6±0,87	712 – 792
	Group B	746,1±0,43	781,6±0,85	
	Group A	739,4±0,44	777,4±0,65	
Protein (g.kg^{-1})	Group C	156,8±0,84	162,2±0,64	156 – 162
	Group B	158,8±0,53	162,8±0,88	
	Group A	162,3±0,78	168,7±0,62	
Fat (g.kg^{-1})	Group C	14,3±0,273	21,1±0,326[a]	37 – 168
	Group B	16,1±0,407	26,7±0,348	
	Group A	20,5±0,581	40,8±0,627[a]	

[1]Reference according Vladau et al., (2008)
[a, b] significant differences between groups: (P<0,05)

When performing the first control catch at the third month, the highest indicator values for moisture were registered for GROUP B 746,1 ± 0,43 g.kg^{-1}, followed by the control group 742,2 ± 0,56 g.kg^{-1} and GROUP A 739,4 ± 0,44 g.kg^{-1}. This trend continued until the end of the study, where again in Group B was observed the highest value of 781,6 ± 0,85 g.kg^{-1}, unlike those in the group with 777,4 ± 0,65 g.kg^{-1} and GROUP A 768,6 ± 0,87 g.kg^{-1}. The values in both control catches were statistically non-significant (P>0,05). A direct impact on the lower moisture content in the meat of fish GROUP B and C, we believe, was performed as a result not only of the type of feed, but also the included feed additive. The results which we received are similar to those of the study performed by

Richter et al., (2003) which report reduced crude fat content and increased moisture content in the meat after using the supplement leaf horseradish tree (*Moringa oleifera*) in the feed. On the other hand, a minimal impact on the chemical composition of the meat, we believe, is performed by the multiplicity of the food, the amount of daily intake and the size of the fish. Such a dependence is established also with the tilapia (*Oreochromis niloticus*) cultured in the recirculation system, where the change of these factors is observed to coincide with a reduced fat deposition and increased moisture content in the meat (El-Zaeem et al., 2012; Antache et al., 2013).

In the indicator crude protein, statistically significant differences were also not considered, both between the groups and within them. Here, however, were confirmed the initial assumptions, so the highest value in the content of crude protein in the meat was found in the fish that has consumed feed with excess protein and fat - GROUP A 168,7 ± 0,62 $g.kg^{-1}$, compared to GROUP B 162,8 ± 0,88 $g.kg^{-1}$ and the control group 162,2 ± 0,64 $g.kg^{-1}$. The increased crude protein content in the meat of the fish from Group A we fully attribute to the higher protein content in the feed, and this assertion is also confirmed by the research performed by Kiaalvandi et al., (2011). They found a direct correlation between the high protein diet in carp feed containing 40% protein content and the subsequently increased crude protein in the meat.

The results obtained by these two indicators are similar to those of the other authors who also tested the effect of different feed additives on the chemical composition of the meat of economically significant species of fish (Cosmin, 2010; Şara et al., 2010). Unlike the previous two indicators, in the raw fats a statistically significant difference was found ($P<0,05$) between Group A and the other two groups. The fat content of the meat in these fish reached a value of 40,8 ± 0,627 $g.kg^{-1}$, which is almost twice higher than in the control group 21,1 ± 0,326 $g.kg^{-1}$. A significant difference was also reported between groups A and Group B ($P<0,05$). The intake of feed with a high fat content (>10% fat) is a constant stimulus for an increased insulin secretion, which in turn stimulates the formation and inhibits the breakdown of fat. As a result of the increased intake of fat, a positive energy balance was observed, which was manifested clinically in the elevation of cholesterol and triglycerides. This phenomenon was also observed in our study, where the values of cholesterol and triglycerides in the blood plasma of carp from group A were the highest.

Table2

Biochemical parameters of blood serum in different groups

Parameter	Group	3rd month	6th month	9th month	Reference[1]
TG ($mg.dl^{-1}$)	Group C	344±50,0	372±39,1[a]	473±40,7[b]	317 – 412
	Group B	360±32,0	547±32,3	561±29,5	
	Group A	408±21,4*	680±28,7[a]	861±31,1[b]*	
HL ($mg.dl^{-1}$)	Group C	261±31,4	264±27,3	285±28,4	99 – 263
	Group B	275±27,1	319±28,2	344±31,5	
	Group A	280±31,9	410±32,1	423±29,8	

[1]Reference according Nicula et al., (2010)
[a, b] significant differences between groups: ($P<0,05$)
*significant differences between basal levels: ($P<0,05$)

Despite the fact that group B was receiving the same kind and quantity of feed but with the addition of Vitas, in it the concentration of cholesterol and triglycerides in the blood and the fat content in the meat were significantly lower (Table 2).

In the literature there are data showing that the plasma levels of triglycerides increase due to the increased "*de novo*" hepatic synthesis of fatty acids, an increase in the activity of the enzyme hydroxymethyl-glutaryl-CoA reductase and the secretion of low density lipoproteins (LDL) as a result of lipoproteins of low density (Feingold et al., 1993). Acceleration of the lipolysis in combination with a reduction in the storage of fatty acids

and the oxidation in the muscles and the fat tissue lead to an increased flow of fatty acids in the liver, and thus an increase in serum triglyceride levels (Zaprianova, 2013). These results are similar to those of other studies related to the reduction in the concentration of triglycerides in the blood after oral intake of silymarin by fish and rats (Fallah Huseini et al. 2006; Ahmadi et al., 2012).

According to some authors, the intake of silymarin inhibits the absorption of cholesterol, thereby it regulates its plasma concentration and cell membrane permeability (Fallah Huseini et al., 2006). It also has a positive impact on the type and amount of membrane lipids (cholesterol and phospholipids) (Basiglio et al., 2009), by inhibiting the cholesterol acyltransferase activity (Sobolová et al., 2006). On the other hand, lower plasma levels can also be the result of lower intestinal reabsorption cholesterol (Tous et al., 2005).

CONCLUSION

The chemical analysis of the meat of fish fed with different rations proved that by increasing the content of protein and fat in the feed statistically significant differences in the chemical composition of the meat are reported. The fundamental rule was confirmed that systematically increased food intake is the most common reason for an increase in the number and size of fat cells. Crucial for the development of this process is the type and quantity of food. As a feed additive, Vitas was used for the first time in fish; there are no data in the literature of its impact on the individual performance of the chemical composition of the meat. But our results, which fully correspond to the data on birds, published by Schiavone et al., (2007), Suchý et al., (2007), Fisinin et al., (2011) gives us reason to conclude that a certain decrease in the crude fat content in the meat is the result of the incorporation of the feed additive Vitas in the rations of carp.

BIBLIOGRAPHY

1.**Adhikari, M., Arora R., Chawla R., Sharma J., Dhaker A., Gupta D., Dubey N., Kumar R., Ivanov V., Gadjeva V., Gevrenova R., Sharma R.,** 2010. *Evaluation of Silymarin as a promising radio protector*. Z. Naturforsch, vol. 65, 337-346.

2. **Adhikari, M., Ivanov V., Adhikary J.S., Karamalakova Y., Zheleva T., Kumar R., Sharma R.K., Gadjeva V., Arora R.,** 2012. *Silymarin can be used as a safe radiation countermeasure in the event of radiological emergencies: Evidence from in vitro and in vivo studies.* Journal of Cancer Research and Therapeutics, vol. 8, 3, 487-488.

3. **Adhikari, M., Dhaker, A., Adhikari, J., Ivanov, V., Singh, V., Chawla, R., Kumar, R., Sharma R., Karamalakova, Y., Gadjeva, V., Arora, R.,** 2013. *In vitro studies on radioprotective efficacy of silymarin against γ-irradiation*. International Journal of Radiation Biology, vol. 89, 3, 200-211.

4. **Ahmadi K., Banaee, M., Vosoghei, A., Mirvaghefei, A., Ataeimehr, B.,** 2012. *Evaluation of the immunomodulatory effects of silymarin extract (Silybum marianum) on some immune parameters of rainbow trout (Oncorhynchus mykiss) (Actinopterygii: Salmoniformes: Salmonidae)*. Acta Ichthyol. Piscat, vol. 42, 2, 113-120.

5. **Alishahi, M., Soltani, M., Mesbah, M., Esmaeilli,A.,** 2011. *Effect of dietary silybum marianum extraction immune parameters of the common carp*. Journal of Veterinary Research, vol. 66, 3, 255-263.

6. **Antache, A., Cristea, V., Grecu, I., Dediu, L., Mocanu, M., Placinta, S., Petrea, S.,** 2013. *The Effects of some phytobiotics on biochemical composition of (Oreochromis niloticus) meat reared in a recirculating aquaculture system.* Animal Science and Biotechnologies, vol. 46, 1, 238-243.

7. **Arora, R., Adhikari, M., Agarwal, P., Chlawa, R., Gupta, D., Sharma, R., Ivanov, V., Karamalakova, Y., Zheleva, A., Gadjeva, V., Stoev, S.,** 2014. *Amelioration of γ-radiation-induced genotoxicity by nanosilymarin: a comparative study indicates possible implications*

for chemical biological radiological and nuclear (CBRN) defence. Trakia Journal of science, vol. 12, 1, 1-10.
8. **Atanasov, A., Zhelev, Z., Ivanov, V., Nikolov, G.,** 2011. *Effects of dietary VITASIL® on the growth performance of Carp (Cyprinus caprio) cultivated in a recirculation system.* Bildiriler Kitabi II, 551-554.
9. **Atanasoff, A., Ivanov, V., Nikolov, G., Zhelyazkov, G., Petrova, B.,** 2012. *Effects of dietary VITASIL® on growth performance of carp (Cyprinus caprio).* Proceeding of days of veterinary medicine, 90-93. ISBN: 978-9989-774-20-X.
10. **Banaee, M., Sureda, A., Ali Reza, M., Reza Rafei, G.,** 2011. *Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (Oncorhynchus mykiss).* Fish Physiol Biochem, vol. 37, 4, 885-896.
11. **Banaee, M., Sureda, A., Shahaf, S., Fazilat, N.,** 2015. *Protective effects of silymarin extract on Malthion-induced Zebra Cichlid (Cichlasoma Nigrofasciatum) hepatotoxicity*. Iranian Journal of Toxicology, vol. 9, 28, 1239-1246.
12. **Basiglio, C., Sánchez Pozzi, E., Mottino, A., Roma, M.,** 2009. *Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis*. Chem. Biol. Interact., vol. 179, 297-303.
13. **Cosmin, B.,** 2010. *The use of fodder additives in some fish species alimentation*. PhD Thesis – University of Agriculture and Veterinary medicine, Cluj-Napoca, Romania
14. **Çağıltay, F., Atanasoff, A., Roydev, R., Zapryanova, D., Bozakova, N., Ivanov, V.,** 2015. *Comparison of effects of clove oil and electro anesthesia on serum biochemical parameters in carp* (*Cyprinus carpio*). Sylwan, vol. 159, 1, 449-460.
15. **D'Souza, D., Mullan B.**, 2002. *The effect of genotype, sex and management strategy on the eating quality of pork*. Meat Science, vol. 60, 1, 95-101.
16. **Dan, Y., Lingfang, G., Binying, D., Ming, L., Yongqing, H., Lei, W., Joshua, G.,** 2012. *Effects of dietary Silymarin supplementation on growth performance and oxidative status in Carassius auratus gibelio.* Journal of Animal and Veterinary Advances, vol. 11, 3399-3404.
17. **El-Zaeem, S., Mohamed, M., Mohamed, E., Abd El-Kader, W.,** 2012. *Flesh quality differentiation of wild and cultured Nile tilapia (Oreochromis niloticus) populations.* African Journal of Biotechnology, vol. 11, 17, 4086-4089.
18. **Fallah, H., Larijani, B., Heshmat, R., Fakhrzadeh, H., Radjabipour, B., Toliat, T., Raza, M.,** 2006. *The efficacy of Silybum marianum Gaertn (Silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial*. Phytother. Res., vol. 20, 12, 1036-1039.
19. **Feingold, K., Hardardottir, I., Memon, R., Krul, E., Moser, A., Taylor, J., Grunfeld, C.,** 1993. *Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters*. J. Lipid Res., vol. 34, 12, 2147-2158.
20. **Fisinin, V., Egorov, I., Andrianova, E., Morina, E., Fel'dman, N., Lutsenko, E., Lutsenko, S.,** 2011. *Influence of liposomal nanoform of flavolignan complex from Silybum marianum L. (Silymarin) on main zootechnical and physiological parameters in broiler-chicken.* Agricultural Biology, vol. 4, 30-35.
21. **Kiaalvandi, S., Faramarzi, M., Iranshahi, F., Zabihi, A., Roozbehfar R.,** 2011. *Influence of feeding frequency on growth factors and body composition of common carp (Cyprinus carpio)fish.* Global Veterinaria, vol. 6, 6, 514-518.
22. **Koynarski, T., Atanasov, A., Sotirov, L.,** 2014. *Influence of VitaSil® additive to carp fish natural immune factors.* Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine, vol. 71, 2, 357-361.
23. **Liem, D.,** 2004. *E. coli resistant to most antibiotics in Vietnam*. Asian Pork. vol. 8, 9, 22-24.
24. **Luper, S.,** 1998*. A review of plants used in the treatment of liver disease*: Part 1. Altern Med Rev, vol. 3, 410-421.

25. **Nicula, M., Bura, M., Simiz, F., Banatean-Dunea, I., Patruica, S., Marcu, A., Lunca, M., Szelei, Z.,** 2010. *Researches concerning reference values assessment of serum biochemical parameters in some fish species from Acipenseridae, Cyprinidae, Esocidae and Salmonidae family*. Animal Science and Biotechnologies, vol. 43, 1, 498-505.
26. **Richter, N., Siddhuraju, P., Becker, K.,** 2003. *Evaluation of nutritional quality of moringa (Moringa opeieralam) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus)*. Aqua, vol. 3, 599-611.
27. **Schiavone, A., Righi, F., Quarantelli, A., Bruni, R., Serventi, P., Fusari A.,** 2007. *Use ofSilybum marianum fruit extract in broiler chicken nutrition: influence on performance and meat quality*. J Anim Physiol Anim Nutr., vol. 91, 5-6, 256-62.
28. **Sobolová, L., Skottová, N., Večeřa, R., Urbánek, K.,** 2006. *Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats*. Pharmacol. Res., vol. 53, 104-112.
29.**Suchý, P., Straková, E., Kummer, V., Herzig, I., Písaříková, V., Blechová, R., Mašková, J.,** 2008. *Hepatoprotective effects of Milk Thistle (Silybum marianum) seed cakes during the chicken broiler fattening*. Acta Vet. Brno, vol. 77,1, 31-38.
30.**Şara, A., Barbu, A., Ani, A., Benţea, M.,** 2010. *The effects of some additives on the bioproductive indices and meat quality of brook trout (Salvelinus fontinalis M.).* Animal Science and Biotechnologies, vol. 43, 1, 94-99.
31. **Tous, M., Ferré, N., Camps, J., Riu, F., Joven, J.,** 2005. *Feeding apolipoprotein E-knockout mice with cholesterol and fat enriched diets may be a model of non-alcoholic steatohepatitis*. Molecular and Cellular Biochemistry, vol. 268, 53-58.
32. **Vladau, V., Bud, I., Stefan, R.,** 2008. *Nutritive value of fish meat comparative to some animal meat*. Animal Science and Biotechnologies, vol. 1, 2, 65-68.
33. **Zapryanova, D.,** 2013. *A study on the experimentally induced inflammation of infectious and non-infectious nature on some aspects of APR and biochemical profile of blood in dogs*.PhD Thesis – Trakia University, Stara Zagora, Bulgaria.