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Ebru Yılmaz [1]
Sevdan Yılmaz [2]
Sebahattin Ergün [2]
Hasan Kaya [3]
Bayram Kızılkaya [2]
Nergiz Soytaş [2]

Authors' addresses:
[1] Bozdogan Vocational School, Adnan Menderes University, Aydın, Turkey.
[2] Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.
[3] Department of Basic Science, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

Correspondence:
Sevdan Yılmaz
Department of Aquaculture, Faculty of Marine Sciences and Technology,
Çanakkale Onsekiz Mart University,
17100 Çanakkale, Turkey.
Tel.: +90 286 2180018 ext. 1589
e-mail: sevdanyilmaz@comu.edu.tr

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A preliminary study of the effect of phytoadditive carvacrol on the trace elements (Cu, Mn and Zn) content in fish tissues

ABSTRACT

Phytoadditives have gained increasing interest as feed additives for fish. The aim of the present study was to determine whether selected dietary phytoadditive can influence the bioavailability of several trace elements (Cu, Mn and Zn), which play an important role in the physiological processes. The experiments were carried out at a commercial trout farm. A total of 420 juvenile rainbow trout (mean weight ± SD = 10.79±0.57), *Oncorhynchus mykiss*, were randomly allocated into four different treatments with three replicates each. Fish were kept in raceways (3X0.8X0.4 m) at 10±1°C with a natural photoperiod. Proper amount of carvacrol was sprayed on 1 kg of commercial trout diet to prepare four diets with 0 (Control, C0), 1 (C1), 3 (C3) and 5 (C5) carvacrol g/kg diet. Fish were fed to apparent satiation three times per day. The feeding trial lasted four weeks. Then, in different type of fish tissues (muscle, liver and pyloric caeca) from fish fed with diets enriched in carvacrol, beneficial elements (Cu, Mn and Zn) were analysed by atomic adsorption spectrophotometer. Results showed that the levels of Cu, Zn and Mn were especially significantly increase by C1 diet in all tissues (muscle, liver and pyloric caeca) except muscle and pyloric caeca Zn. The results of this experiment indicate that the carvacrol had the ability to potentiate the trace element retention. Although bioaccumulations of Cu, Zn and Mn in the muscle, liver and pyloric caeca are well demonstrated, the exact mechanisms of phytoadditives are still only partially understood. More investigations are required to detail the mechanisms involved in phytoadditives this enhancement.

Key words: phytoadditive, carvacrol, trace elements, Cu, Mn, Zn, *Oncorhynchus mykiss*

Introduction

Trace elements constitute a relatively small amount of the total body tissues (Mumtaz et al., 1999). However, they are essential for normal life processes of fish (Lim et al., 2001). The functions of the trace elements vary considerably. They are generally involved in the regulation of very diverse cellular metabolic roles and they may act as cofactors in biochemical reactions (Jobling, 2001).

Copper (Cu) is a cofactor for some enzyme systems used in energy metabolism, and is essential for normal functioning of the brain, skeleton and spinal cord (Webster & Lim, 2002). The Cu metalloenzymes are involved in cellular energy production, protection of cells from free radical damage, brain neurotransmitters, collagen synthesis, and melanin production (Lall, 2002).

Manganese (Mn) is involved in the formation of bone, blood clotting, insulin function and cholesterol synthesis (Webster & Lim, 2002), and its functions either as a cofactor activating a large number of enzymes that form metal–enzyme complexes or as an integral part of certain metalloenzymes in carbohydrate, lipid, and protein metabolism (Lall, 2002).

Zinc (Zn) is required for normal growth, development, and function of all animal species and the major function of zinc in fish metabolism is as a cofactor for numerous enzymes (Lim et al., 2001). Zn is also required for normal bone calcification, in the transfer of carbon dioxide in red blood cells, and for the synthesis and metabolism of proteins and nucleic acids (Webster & Lim 2002).

For their aforementioned properties, Cu, Zn and Mn are important for the fish health. Phytoadditives are fodder additives obtained from spices, medicinal plants or plants extract. They have been reported to promote various functions like growth (Shalaby et al., 2006; Yılmaz & Ergün, 2013), appetite stimulation, antistress properties (Citarasu, 2010), immune functions (Dügenci et al., 2003; Dorucu et al., 2009; Ergün et al., 2011), pigmentation (Yılmaz & Ergün, 2011; Yılmaz & Ergün, 2013), protein and energy retentions (Yılmaz et al., 2012a), hematological and biochemical status (Yılmaz & Ergün, 2012) and also increase disease resistance in fish (Yılmaz et al., 2012b; Yılmaz et al., 2013).

On the other hand, the effects of dietary phytoadditives on the bioavailability of trace elements are not clear. Hitherto, there was only one report on the effects of phytoadditive (garlic+ginger or oregano+echinacea) on bioavailability of trace element (Se, selenium) in fish (Gabor et. al., 2012).

Carvacrol is a major component of oregano and thyme essential oils. It is recognized as a safe component by the U.S. Food and Drug Administration (2010), by the Council of Europe (2000), and FAO/WHO Committee on Food Additives (2001). Several studies have reported that oral administration of carvacrol in *Ictalurus punctatus* (Zheng et al., 2009; Rattanachaikunsopon & Phumkhachorn 2010), *Oncorhynchus mykiss* (Ahmadifar et al., 2011; Giannenas et al., 2012), and *Dicentrarchus labrax* (Volpatti et al., 2012) improved growth performance, disease resistance and/or immunity.

The aim of the present study was to determine whether selected dietary phytoadditive can influence the bioavailability of several trace elements (Cu, Mn and Zn), which play an important role in the physiological processes.

Materials and Methods

Fish and experimental conditions

The experiments were carried out at a commercial trout farm (Çobanlar) in Muğla province, Turkey. A total of 420 juvenile rainbow trout (mean weight ± SD = 10.79±0.57), *Oncorhynchus mykiss*, were randomly allocated into four different treatments with three replicates each. Fish were kept in raceways (3x0.8x0.4 m) at 10±1°C with a natural photoperiod. Water dissolved oxygen was maintained between 7.7 and 7.5 mg/L. The raceways had continuous water flow with a turnover rate of 6 to 8 times per day.

Experimental diets, design and feeding trial

Proper amount of carvacrol was sprayed on 1 kg of commercial trout diet (Çamlı BioAqua, Table 1) to prepare four diets with 0 (Control, C0), 1 (C1), 3 (C3) and 5 (C5) carvacrol g/kg diet. Fish were fed to apparent satiation three times per day. The feeding trial lasted four weeks.

Table 1. *Chemical analysis of landfarming soil.*

Chemical analyses	
Crude protein (%)	49
Crude lipid (%)	19
Crude cellulose (%)	3
Crude ash (%)	13
Energy (kcal/kg)	4329
Amino acids (%)	
Lysine	4.7
Methionine + Cystine	2.4
Vitamins (per kg feed)	
A (IU)	2500
D3 (IU)	3050
E (mg)	240
K (mg)	10
C (mg)	250
Macro elements (%)	
Calcium	1–2
Total phosphor	1.5
Sodium	0.2/1
**Trace elements (µg/g)*	
Zn	160.77±4.24
Mn	54.63±1.07
Cu	12.00±0.58

Legend: Ingredients: Fish meal, fish oil, soybean and by products, wheat and by products, yeast and by products, amino acids, vitamins and minerals.
* Values are mean ±SEM; n=3, trace elements analysis was carried out by atomic adsorption spectrophotometer (AA6300, Shimadzu, Japan).

Tissue trace element analysis

Tissues (liver, muscle and pyloric caeca) and commercial trout diet for trace metal analysis were oven dried to a constant weight, digested in 5 ml of concentrated nitric acid, then diluted to 20 ml with deionised water (Smith et al., 2007). Then, Cu, Mn and Zn analysis was carried out by atomic adsorption spectrophotometer (AA6300, Shimadzu, Japan).

Statistics

Each value was expressed as mean ± SEM for each of the measured variables. The statistical significance ($P < 0.05$) of tissue trace elements were tested using Student's *t*-test with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software.

Results

Trace elements content in muscle tissues is shown in Table 2. The muscle Cu and Mn levels in fish fed with C1 supplementation diet was significantly increased than the control diet. However, C3 supplementation did not significantly change muscle Cu, Mn and Zn from control values. In addition, C1 and C5 supplementations resulted in a significantly reduced muscle Zn content.

Table 2. *Concentrations (µg/g dry wt) of Cu, Mn and Zn in muscle of rainbow trout fed control (C0) or carvacrol supplemented diets (C1= carvacrol at 1g/kg, C3= carvacrol at 3g/kg, C5= carvacrol at 5 g/kg) for 30 days.*

	Cu	Mn	Zn
C0	1.87±0.12	7.44±0.96	51.54±2.42
C1	4.29±0.64*	17.79±2.08*	16.84±1.89*
C3	1.54±0.19	4.60±0.94	53.78±10.99
C5	1.84±0.19	5.93±0.93	21.67±2.82*

Legend: Asterisks indicate significant differences between control and treatment groups ($P<0.05$). Values are mean ±SEM; n=9.

Trace elements content in liver tissues is shown in Table 3. Liver Cu and Zn levels in fish that were fed the C1, C3 and C5 diets were significantly higher than the control values. Liver Mn levels were also significantly higher in fish that were fed with C1 and C3 diets compared with the control diet.

Trace elements content in pyloric caeca tissues is shown in Table 4. The pyloric caeca Cu levels in fish fed with C1, C3 and C5 supplementation diets were significantly increased than the control diet. Mn levels in the fish pyloric caeca that were fed with C1 and C3 diets were significantly higher than the control values. The Zn levels in the C3 treatment groups were higher than the control level, whereas Zn in the C5 treatment group was lower than that in the control group.

Table 3. *Concentrations (µg/g dry wt) of Cu, Mn and Zn in liver of rainbow trout fed control (C0) or carvacrol supplemented diets (C1= carvacrol at 1g/kg, C3= carvacrol at 3g/kg, C5= carvacrol at 5 g/kg) for 30 days.*

	Cu	Mn	Zn
C0	18.44±2.53	3.44±0.32	53.24±1.73
C1	107.76±2.90*	17.94±1.86*	135.18±11.40*
C3	41.88±2.33*	5.79±0.67*	85.70±9.56*
C5	76.75±3.10*	5.34±0.61	67.14±1.59*

Legend: Asterisks indicate significant differences between control and treatment groups ($P<0.05$). Values are mean ±SEM; n=9.

Table 4. *Concentrations (µg/g dry wt) of Cu, Mn and Zn in pyloric caeca of rainbow trout fed control (C0) or carvacrol supplemented diets (C1= carvacrol at 1g/kg, C3= carvacrol at 3g/kg, C5= carvacrol at 5 g/kg) for 30 days.*

	Cu	Mn	Zn
C0	34.06±2.60	4.01±0.85	168.75±13.00
C1	139.46±0.65*	9.32±0.56*	130.97±4.84
C3	104.19±12.79*	11.89±1.07*	240.52±19.54*
C5	89.39±13.33*	3.97±0.54	106.94±6.09*

Legend: Asterisks indicate significant differences between control and treatment groups ($P<0.05$). Values are mean ±SEM; n=9.

Discussion

The phytoadditives act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes in animals (Wenk, 2003a). The consequence can be a beter nutrient utilization and absorption (Wenk, 2003b) or improving growth performance and immune system (Wenk, 2003c). In addition, for many essential elements, there is a range of tissue levels compatible with optimum growth and function. It is also known that essential elements are implicated in many physiological processes contributing to the body's natural defenses (Maggini et al., 2007).

Previous studies reported that the carvacrol or thymol-carvacrol powder have been reported to promote various health functions like growth (Zheng et al., 2009; Ahmadifar et al., 2011) antioxidant protective capacities (Giennenas et

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al., 2012) and also increased disease resistance in fish (Zheng et al., 2009; Rattanachaikunsopon & Phumkhachorn 2010; Volpatti et al., 2012).

Stef and Gergen (2012) evaluated the use of Lemon balm, Sage, St. John's wort and Small-flowered Willowherb in a mineral-enriched containing 2% for chicken 43 day, and they stated that 2% sage enhanced accumulation of Cu, Fe, Mn and Zn in all tissues (liver, leg meat and breast meat). Taranu et al. (2012) also reported that dietary supplementations of essential oil mixture containing 25 g limonene, 5 g eugenol and 12 g pinene per kg product (0.04%) significantly increased the level of cytokine (IL-1β, IL-8, TNF-α, IFN-γ) production in liver within the 11 days in piglets. They declared that these findings revealed an improvement in mineral (Cu and Fe) stores in liver, produced by essential oils.

Nevertheless, information on the effect of phytoadditives, on mineral utilization and absorption in fish is poorly understood. Gabor et. al. (2012) conducted an experiment with *O. mykiss* fed a basal diet containing 2% garlic + 1% ginger mix and 1% oregano + 0.5% Echinacea mix for 95 days, and they found that the use of garlic+ginger or oregano+echinacea improved bioavailability of Se. However, no study has been reported in literature with respect to the dietary carvacrol on the mineral utilization and absorption of fish. In this study, results showed that the levels of Cu, Zn and Mn were especially significantly increase by C1 diet in all tissues except muscle Zn.

The results of this experiment indicate that the carvacrol had the ability to potentiate the trace element retention. Although bioaccumulations of Cu, Zn and Mn in the muscle, liver and pyloric caeca are well demonstrated, the exact mechanisms of phytoadditives are still only partially understood. More investigations are required to detail the mechanisms involved in phytoadditives this enhancement.

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